EPA Registration Number 86833-1

ISB'S Front-end PRIA Completeness Screen

Draft 3; 10/25/07

EPA Receipt Date: 9/17/09		EPA Reg. Number: 86833-R		
	Check List Item	Yes	No	N/A
1	Has the **PRIA Fee been Paid**; is a copy of the check or Pay.gov receipt included in the Submission Package?		✓	
2	Is an **Application Form** (EPA Form 8570-1) Included in the Submission Package, is it completely filled out and signed including package type?	✓		
3	Is a **Confidential Statement of Formula** (EPA Form 8570-29) Included in the Submission Package, is it completely filled out and signed (boxes 1-21)?	✓		
4	Is a **Formulator's Exemption Statement** (EPA Form 8570-27) Included in the Submission Package?		✓	
5	Is a **Certification with Respect to Citation of Data** (EPA Form 8570-34) Included in the Submission Package?		✓	
6	Is a **Data Matrix** (EPA Form 8570-35) Included in the Submission Package?	✓		
7	Is a **Label** Included in the Submission Package?	✓		
8	Are **Data** Included in the Submission Package?	✓		
9	**Is the Submission an Amendment?**		✓	

Material Sent for Data Extraction

Reg. # 86833-1

Description: New Registration

☐ Material(s) Sent to Data Extraction Contractors:

- ☒ New Stamped Label Dated 1/30/12
- ☐ Notification Dated _____
- ☐ New CSF(s) Dated _____
- ☒ Other: New Registration

☐ Decision #: 420440

☐ Other Action/Comments: _____

_____

File this coversheet and attached materials in the jacket. It must be well organized and clipped together, NOT STAPLED. Then give the jacket with the coversheet and materials to staff in the Information Services Center (ISC) (Room S-4900). If a jacket is full or only available as an image, please file materials in a new jacket and bring it down to the (ISC). For further information please call 703-605-0716.

Reviewer: Jennifer Gaines

Phone: 305-5967 Division: RD

Date: 2/22/12 2/22/12

U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Pesticide Programs
Registration Division (7505P)
1200 Pennsylvania Ave., N.W.
Washington, D.C. 20460

EPA Reg. Number:	Date of Issuance:
86833-1	JAN 30 2012

NOTICE OF PESTICIDE:
_X_ Registration
____ Reregistration
(under FIFRA, as amended)

Terms of Issuance:

Unconditional

ZonaStat-H

Name and Address of Registrant (include ZIP Code):

Humane Society of the United States
c/o Morgan, Lewis & Bockius LLP
1111 Pennsylvania Ave., NW
Washington, DC 20004

Attention: Kathleen M. Sanzo

Note: Changes in labeling differing in substance from that accepted in connection with this registration must be submitted to and accepted by the Registration Division prior to use of the label in commerce. In any correspondence on this product always refer to the above EPA registration number.

On the basis of information furnished by the registrant, the above named pesticide is hereby registered/reregistered under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA).

Registration is in no way to be construed as an endorsement or recommendation of this product by the Agency. In order to protect health and the environment, the Administrator, on his motion, may at any time suspend or cancel the registration of a pesticide in accordance with the Act. The acceptance of any name in connection with the registration of a product under this Act is not to be construed as giving the registrant a right to exclusive use of the name or to its use if it has been covered by others.

This product is unconditionally registered in accordance with FIFRA section 3(c)(5) provided that you:

Signature of Approving Official:	Date:
John Hebert Product Manager (07) Insecticide-Rodenticide Branch Registration Division (7505P)	JAN 30 2012

EPA Form 8570-6

Notice of Registration (continued)
EPA Reg. No. 86833-1

A. Submit and/or cite all data required for registration/reregistration of your product when the Agency requires all registrants of similar products to submit such data.

B. Make the following label changes before you release the product for shipment:

1. Revise your registration number to "EPA Reg. No. 86833-1",

2. Add "Department of Interior, and all its designated agents" before "National Park Service..." located in the first bullet of the certified applicators section,

3. Add a bullet in the certified applicators section that reads "USDA and all its designated agents (i.e., U.S. Forest Service, Animal and Plant Health Inspection Service),

4. Delete "Federal Land Management Agency" as there is no such entity,

5. Remove "Draft" from "Draft Package Insert for Zonastat-H", "DRAFT LABEL FOR ZONASTAT-H BAG", and "DRAFT LABEL FOR ZONASTAT-H VIAL".

6. Place a box around the restricted use section.

7. Revise "SEE OTHER PANEL FOR..." to read "SEE BACK PANEL FOR PRECAUTIONARY STATEMENTS".

8. Add an "S" to ACTIVE INGREDIENT.

9. Under "Other ingredients" add "Total" and then add "100%" under "99.9%".

10. On page 4, change "Avoid contact with eyes" to read "Do not contact with eyes."

11. Revise the second sentence under the section title Application Rate to read "Efficacy is maintained by annual booster doses."

12. Revise "SEE PACKAGE INSERT FOR PRECAUTIONARY STATEMENTS" found on the label for the bag and vial to read "SEE PACKAGE INSERT FOR PRECAUTIONARY STATEMENTS AND DIRECTIONS FOR USE".

13. Remove "Store loaded darts in a cool dry area. In humid areas of the country, store in plastic sealable bags with a desiccant." from the Pesticide Storage paragraph of the Storage and Disposal section,

14. Add a reference to dart in the Pesticide Disposal section,

15. Create a second paragraph in the Pesticide Storage section that reads:

> "Storage: The vials containing PZP solution are stored frozen. The frozen PZP solution expires two years after freezing. After defrosting, the PZP solution expires after 24 hours. When transporting for use in the field, store the PZP solution in a cooler with ice packs. If transportation of the PZP solution takes longer than 8 hours, store the PZP solution on dry ice in the cooler. Store loaded darts in a cool dry place. In humid areas of the country, store in plastic sealable bags with a desiccant."

C. Submit one (1) copy of final printed labeling for the record before you release the product for shipment.

A copy of your label stamped "Accepted with Comments in EPA Letter Dated January 30, 2012" is enclosed for your records.

Reference to Website on Label

Should you wish to add a reference to the company's website on your label, please be aware that the website becomes labeling under the Federal Insecticide Fungicide and Rodenticide Act and is subject to review by the Agency. If the website is false or misleading, the product would be misbranded and unlawful to sell or distribute under FIFRA section 12(a)(1)(E). 40 CFR 156.10(a)(5) list examples of statements EPA may consider false or misleading. In addition, **regardless of whether a website is referenced on your product's label**, claims made on the website may not substantially differ from those claims approved through the registration process. Therefore should the Agency find or if it is brought to our attention that a website contains false or misleading statements or claims substantially differing from the EPA approved registration, the website will be referred to the EPA's Office of Enforcement and Compliance Assurance.

Confidential Statement of Formula

The acceptable Confidential Statement of Formula (CSF) for this product is dated September 12, 2011 (Basic Formulation). All other versions are obsolete.

If these conditions are not complied with, the registration will be subject to cancellation in accordance with FIFRA section 6(e). Your release for shipment of the product constitutes acceptance of these conditions.

If you have any questions or comments regarding this letter, please contact Jennifer Gaines by phone (703-305-5967) or e-mail (gaines.jennifer@epa.gov).

Enclosures: 1. Stamped Label

DRAFT PACKAGE INSERT FOR ZONASTAT-H

ACCEPTED
with COMMENTS
In EPA Letter Dated
JAN 30 2012
Under the Federal Insecticide, Fungicide, and Rodenticide Act, as amended, for the pesticide registered under EPA Reg. No. 81633-1

FRONT PANEL

RESTRICTED USE PESTICIDE

For retail sale to and use by Certified Applicators or persons under their direct supervision of the following organizations and their designated wildlife management personnel and only for those uses covered by the Certified Applicators certification:

- **National Park Service, Bureau of Land Management, U.S. Fish & Wildlife Service, and Federal Land Management Agency**
- **State departments of agriculture/livestock and wildlife, and their designated agents**
- **Federally recognized Indian tribes, and their designated agents**
- **Department of Defense and its designated agents**
- **Public and private wild horse sanctuaries and reserves**
- **Humane Society of the United States and designated agents.**

Each Responsible Authority for wild horses and/or burros intended to be treated with ZONASTAT-H must sign a certification of use prior to the administration of the vaccine to any animals. The certification statement is attached to this Package Insert.

ZONASTAT-H

Product information: ZonaStat-H is a porcine zona pellucida immunocontraceptive vaccine indicated for use in limiting the populations of wild and feral horses (*Equus caballus*) and burros (*Equus asinus*).

KEEP OUT OF REACH OF CHILDREN

CAUTION

FIRST AID
HAVE LABEL WITH YOU WHEN OBTAINING TREATMENT ADVICE

If on skin: Take off contaminated clothing.
Rinse skin immediately with plenty of water for 15-20 minutes.
Call a poison control center or doctor for treatment advice.

If inhaled: Move the person to fresh air.
If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably mouth-to-mouth if possible.
Call a poison control center or doctor for treatment advice.

DB1/ 67193892.2

If in eyes: Hold eye open and rinse slowly and gently with water for 15-20 minutes.
Remove contact lenses, if present, after the first 5 minutes, then continue rinsing.
Call a poison control center or doctor for treatment advice.

Have the product container or label with you when calling a poison control center or doctor or going for treatment. You may also contact 1-800-xxx-xxxx for emergency medical treatment information.

SEE OTHER PANEL FOR PRECAUTIONARY STATEMENTS

RESTRICTED USE PESTICIDE

ZONASTAT-H

ACTIVE INGREDIENT:
Porcine zona pellucida (ZP3)(0.1%)..... 0.071%
Porcine zona pellucida (ZP1, ZP2, ZP4)(0.1%).....0.029%

Other ingredients..... 99.9%

Batch Code:

EPA Registration No. TBD

If pregnant, take precaution when preparing, loading, and recovering darts to not self-inject.

Humane Society of the United States
2100 L Street NW
Washington, DC 20037
202-452-1100

Net Contents 0.5 mL
Single dose

BACK PANEL

PRECAUTIONARY STATEMENTS

HAZARDS TO HUMANS AND DOMESTIC ANIMALS

Keep away from humans, domestic animals, and pets. Wear protective gloves when handling. If pregnant, take necessary precautions when preparing, loading, and recovering darts to not self-inject. Accidental injection may cause infertility in women. Wash all implements used for handling or applying product with detergent and water. Do not use these implements for mixing, holding, or transferring food or feed.

In the event of an accidental needle stick or cut, clean wound immediately with soapy water and disinfect wound with alcohol or other bactericidal solution. In the event of accidental contact with Modified Freund's Complete Adjuvant, wipe skin clean with an ethanol soaked towelette and wash with soapy water.

ENVIRONMENTAL HAZARDS

Do not apply this product directly to water, or to areas where surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water by cleaning of equipment or disposal of wastes.

PERSONAL PROTECTIVE EQUIPMENT (PPE)

Applicators and other handlers must wear:

- long sleeved shirt and long pants
- gloves
- shoes plus socks

USE INFORMATION

When injected into a female horse or burro, ZonaStat-H stimulates the production of anti-zona pellucida (ZP) antibodies. These antibodies bind to the native ZP glycoproteins surrounding the egg of the target female, alter their conformation, and block sperm attachment, thereby preventing conception.

USE RESTRICTIONS

It is a violation of Federal law to use this product in a manner inconsistent with its labeling. A copy of this label must be in the possession of the user at the time that the product is applied.

READ THIS LABEL: Read this entire label and follow all use directions and precautions.

IMPORTANT: Do not expose children, pets, or other non-target animals to this product. To help prevent accidents:

1) Keep children out of areas where this product is used.
2) Store product in use in a location out of reach of children and pets.
3) Apply product only according to the directions authorized.
4) Dispose of product container and spoiled or unused product as specified in the "STORAGE AND DISPOSAL" section on this label.

ZonaStat-H is for use only in female wild and feral horses and burros, which are defined as free-roaming horses or burros, privately or publicly owned, that are capable of doing environmental damage.

Caution is required to prevent accidental self-injection when administering ZonaStat-H.

Pregnant women should take necessary precautions when preparing , loading , and recovering darts to not self-inject. Do not ingest. Avoid contact with eyes.

Do not apply this product to food or feed. Do not apply ZonaStat-H to horses or burros being used as food.

DIRECTIONS FOR USE

Mixing PZP Solution and Adjuvant:
This activity takes place in the field.

Equipment Needed:
2 BD #2311 glass syringes, 5.0 cc, graduated at 0.2 cc, with Luer-Loc
BD 1.5-inch 18 g disposable sterile needle
Vial of Adjuvant (supplied separately)
Vial of PZP Solution
Luer-Loc connector

FOR HAND DELIVERY
BD 3cc disposable plastic syringe with Luer-Lok
BD 1.5-inch 18 g disposable sterile needle
BACK PANEL (Continued)

FOR JAB-STICK DELIVERY
Dan-Inject® Fiskars Combi-Click Jab Stick
BD 3cc disposable plastic syringe with Luer-Lok
Monoject 1.5-inch 14 g disposable sterile needle

FOR REMOTE (DART) DELIVERY
BD 2.0 inch 18 g disposable sterile needle

1.0-cc C-type or P-type Pneu-Dart dart with 1.25-inch or 1.5-inch barbless needle

Procedures:

1. Examination gloves must be worn during mixing and loading of PZP solution.
2. Attach the Luer-Lok connector to one of the glass syringes.
3. Place the 1.5-inch needle on the second glass syringe.
4. Draw out 0.5 cc of adjuvant (supplied separately).
5. Using the same syringe, draw up the 0.5 cc of PZP in phosphate buffered saline solution.
6. Holding the syringe containing the vaccine very carefully (to prevent the plunger from slipping out), take off the needle and attach the syringe to the second syringe using the Luer-Lok connector.
7. Push the PZP solution-adjuvant mixture back and forth through the two syringes 100 times. The resulting emulsion will become thick and look white. THIS PROCEDURE IS VERY IMPORTANT AND IS RELATED TO THE PRESENTATION OF THE ANTIGEN AND THE SUBSEQUENT EFFICACY OF THE PRODUCT.
8. Make sure that all of the emulsion is in one syringe.
9. Holding the syringe containing the emulsion very carefully, remove the other syringe, leaving the Luer-Lok on the syringe containing the emulsion.

FOR HAND DELIVERY (INJECTION), attach a 2.0 or 3.0cc plastic syringe to the glass syringe via the Luer-Lok, and inject the emulsion into the plastic syringe. After loading the plastic syringe, disconnect the glass syringe and connect an 18 g 1.5-inch needle to the plastic syringe containing the emulsion.

FOR JAB-STICK DELIVERY, place the nose of the plastic syringe tightly into the Luer-Lok and inject the emulsion from the glass syringe into the plastic syringe. After filling the plastic syringe, remove the glass syringe and attach the Monoject 14g 1.5-inch needle to the plastic syringe containing the emulsion. Place the plastic syringe into the jab-stick.

FOR REMOTE (DART) DELIVERY

- Attach the 18 g 2-inch needle to the glass syringe containing the emulsion. Insert the needle into the body of the dart through the dart needle, and inject the contents of the syringe into the dart. Apply a small amount of Vaseline to the dart tip.

- After the antigen solution and adjuvant are emulsified in the field and loaded into the dart, remotely inject ZonaStat-H intramuscularly in the hip or gluteus or hamstring muscles using a syringe dart fired from a CO_2 or cartridge-powered projection system.

- Use the Pneu-Dart 1.0-cc dart with a 1.25-inch or 1.5-inch barbless needle for delivery.

- The darts can be delivered using any of the following rifles, depending on the logistical requirements of the particular targeted population:

  - Dan-Inject® CO_2 rifle (Wildlife Pharmaceuticals) with a 13 mm barrel (for use at ranges of 10 meters to 40 meters)
  - Dan-Inject® Pistol Grip Blow Gun with a 13 mm barrel (for use at ranges of 5-20 meters)
  - Pneu-Dart® model 193 rifle (for use at ranges of up to 50 meters)
  - Pneu-Dart® model 389 cartridge-fired rifle (for use at ranges of up to 50 meters)

- The applicator must make every attempt to recover all darts. If possible, all darts that are discharged and drop from the horse at the shooting site must be recovered before another darting occurs. In exceptional situations, such as an onset of inclement weather, loss of daylight, applicator safety concerns, or other urgent circumstances, the site of a lost dart may be noted and marked, and recovery efforts made at a later time. Examine all fired darts after recovery to determine if the charge fired and the plunger fully expelled its vaccine contents.

Application Rate:

For maximum efficacy, ZonaStat-H is administered as an initial priming dose followed by a booster dose at least two weeks later. Full efficacy is maintained by annual booster doses.

Initial Priming Dose

The initial treatment (priming dose) of ZonaStat-H consists of 0.5 cc of the PZP solution emulsified with 0.5 cc modified Freund's Complete Adjuvant If followed by a booster dose, the priming dose may be administered at any time of the year. The priming dose alone is expected to reduce pregnancy rates by 55-70% for one year if administered one to three months prior to the onset of the mating season.

Booster Dose

A booster dose of ZonaStat-H consists of 0.5 cc of the PZP solution emulsified in 0.5 cc modified Freund's Incomplete Adjuvant. Administration of a single booster treatment at least two weeks after the administration of the priming dose is expected to reduce pregnancy rates by 90-95% for one year. Efficacy in subsequent years is maintained by administering an annual booster dose.

PACKAGING

Packaging:
PZP antigen dissolved in phosphate buffered saline solution is packaged in screw-top, non-refillable plastic vials containing single 0.5 mL doses.

Adjuvant is provided separately for the initial priming dose and the booster dose. The adjuvant is provided in multidose bottles.

STORAGE AND DISPOSAL

Pesticide Storage:
Keep vials of PZP antigen (i.e., PZP + phosphate buffer solution) frozen until ready for use. When transporting for use in the field, keep PZP antigen stored in a cooler, with ice packs If transportation takes longer than 8 hours, store PZP antigen on dry ice in the cooler. Keep adjuvant refrigerated at +2°C to +8°C, but not frozen, until ready to be mixed with the PZP antigen. Store loaded darts in a cool dry area. In humid areas of the country, store in plastic sealable bags with a desiccant.

Pesticide Disposal:
If the PZP solution is not used within 24 hours of defrosting, or if not properly stored while in the field, dispose of unused ZonaStat-H material and loaded syringes as medical waste according to applicable Federal, State, and/or Local regulations.

Container Disposal:
Non-refillable container. Do not reuse or refill container. Dispose of expired material, preloaded syringes, used syringes, darts, and needles as medical waste according to applicable Federal, State, and/or Local regulations.

Storage:
The vials containing PZP solution are stored frozen. The frozen PZP solution expires two years after freezing. After defrosting, the PZP solution expires after 24 hours. When transporting for use in the field, the PZP should be stored in a cooler with ice packs. The cooler should contain dry ice if transportation of the PZP takes longer than 8 hours.

The adjuvant should be refrigerated +2°C to +8°C, but not frozen.

Store loaded darts in a cool dry area. In humid areas of the country, it is recommended that they be stored in plastic sealable bags with a desiccant.

Disposal:
Used darts: Used darts should be disposed of in a sharps container, and further disposed of in accordance with state laws regarding disposal of medical waste.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

DECISION MEMORANDUM

SUBJECT: First U.S. Registration of the Active Ingredient, Porcine Zona Pellucida

FROM: Lois Rossi, Director
Registration Division

TO: Steven Bradbury, PhD., Director
Office of Pesticide Programs

This memorandum recommends that you concur on the first U.S. registration of the contraceptive, porcine zona pellucida, for population control of wild and feral horses and burros. A summary of the human health and ecological risks are included in the attached Registration Decision Document. The Health Effects Division and the Environmental Fate and Ecological Effects Division have reviewed all available data and the Registration Division has concluded that the criteria for unconditional registration under FIFRA Section 3(c)(5) have been met.

RECOMMENDATIONS

I recommend for the unconditional registration of the new chemical, porcine zona pellucida, under FIFRA Section 3(c)(5).

Concur: _____
Steven Bradbury, PhD., Director
Office of Pesticide Programs

Nonconcur: _____
Steve Bradbury, PhD., Director
Office of Pesticide Programs

Attachments: Registration Decision Document and Fact Sheet

Registration of the Contraceptive ZonaStat-H, for Population Control of Wild and Feral Horses and Burros

Approved by: Lois Rossi

Lois Rossi, Director
Registration Division

Date: February 9, 2012

Registration of the New Active Ingredient Porcine Zona Pellucida Formulated as a Contraceptive, "ZonaStat-H," to Aid in the Control of Wild and Feral Horse and Burro Populations

Regulatory Rationale

The U.S. Environmental Protection Agency (hereon referred to as EPA or the Agency) is granting the first registration of a pesticide product containing the active ingredient porcine zona pellucida (PZP).

Wild horses and burros inhabiting private and state-owned lands are the responsibility of state agencies which have to address issues involving these animals when conflicts arise. Federal agencies such as the Department of Interior (DOI), the U.S. Forest Service (USFS), Bureau of Land Management (BLM), and the Department of Defense (DOD) manage lands that support wild horses and burros. The Free-Roaming Wild Horse and Burro Act gives BLM the authority for managing wild horse and burro populations on BLM land. BLM also works with the USFS to manage these populations on national forests land. The National Park Service (NPS) considers wild horses and burros to be an "exotic species" or "feral livestock" and manages herds found on Park lands.

Like other animals (e.g. deer, Canada geese, etc.), horses may be pests in some situations. Expanding populations of feral horses are adversely affecting public and private lands by over-grazing and competing with native populations of animals for food. Wild horse and burro populations that are not managed have the potential to reach densities that damage land and wildlife habitat; conflicts with livestock growers and recreational users may also arise. For example, wild horses on Assateague Island can destabilize and reduce the height of the dunes that protect the island by trampling and grazing intensively on dune grass and salt meadow hay. Additionally, wild horses feeding on western rangelands can overlap with those of cattle, sheep, and elk, and can result in direct competition with these animals for food. Since wild burros inhabit more arid regions, their impacts are seen close to water sources. Wild burros are seen as a threat to natural resources such as the desert tortoise and desert bighorn sheep.

I. Chemical Information

Chemical Name: Porcine Zona Pellucida (PZP)

EPA PC Code: 176603

CAS Number: None

Porcine zona pellucida is a complex of four different acidic glycoproteins, ZP1 (80,000-90,000KD), ZP2 (60,000 – 65,000 KD), ZP3 (55,000 KD), and ZP4 (20,000 – 25,000 KD). PZP consists of approximately 70 – 80% ZP3. To obtain the PZP antigen, porcine ovaries are collected from freshly slaughtered female pigs at USDA-inspected slaughterhouses, and immediately frozen. Oocytes are collected, washed, and then homogenized in a buffered salt

solution. Then the zonae are heat-solubilized in a phosphate buffer solution (PBS) then diluted. The resulting zona pellucida solution is frozen until use. To create the end use product, ZonaStat-H, 100 µg PZP antigen is dissolved in 0.5 mL PBS, which is then emulsified in the field by the applicator in 0.5 mL Modified Freund's Complete Adjuvant (mFCA) or Modfied Freund's Incomplete Adjuvant (mFIA).

Mode of Action: PZP antigen is the glycoprotein layer that surrounds the oocyte and is weakly antigenic by itself. Therefore, PZP is emulsified with an adjuvant (mFCA for the primary vaccination and mFIA for booster vaccinations) which stimulates a stronger immune response. This results in the creation of anti-zona peullucida antibodies which bind to the zona pellucida of the oocyte, alter their conformation, and block sperm attachment to the zona pellucida receptors.

Registrant: Humane Society of the United States (HSUS)

Proposed Product: The proposed ZonaStat-H product (EPA File Symbol 86833-R), is an injectable immunocontraceptive vaccine for use only on female feral and wild horses and burros. As a Restricted Use Pesticide (RUP), ZonaStat-H can only be administered by certified applicators, or by persons under the direct supervision of a certified applicator. ZonaStat-H is injected into the target animals by one of the following three methods: hand deliver, jab-stick delivery, and remote (dart) delivery.

II. Human Health Risk

A summary of the human health effects and risk of PZP as assessed in the Agency document entitled "*ZONASTAT-H. Immunocontraceptive Vaccine for Limiting the Population of Wild and Feral Horses and Burros*" dated July 8, 2010, is provided below.

The HSUS submitted waiver requests for the toxicity studies ordinarily required for a terrestrial, non-food use pesticide. The waiver requests were granted due to lack of toxicity on the target animal; a history of safe use of the vaccine, explained further below; the mode of action and fate of the product's metabolites; the limited opportunity of exposure to non-target animals, applicators, and the public; and lack of immunotoxicity as shown in the published scientific literature.

Under the sponsorship of the National Park Service (NPS), testing of ZonaStat-H began in 1988 on wild horses at Assateague Island National Seashore (ASIS) and it has subsequently been tested on white-tailed deer, zoo animals, African elephants, and other animals. Between 1994 and 2007, 901 female horses were darted with PZP by two applicators without any incident or report of harm to the applicators. Since 2004, ZonaStat-H has been administered to an estimated 1800 western wild horses on 47 herd management areas by researchers and Bureau of Land Management personnel. Also, 136 zoos have administered ZonaStat-H to captive animals with no reports of harm or adverse effects to applicators.

The basic biology and properties of ZonaStat-H, i.e., its mechanism of action - ZonaStat-H contains porcine zona pellucida antigen (the glycoprotein layer surrounding the oocyte) and an

adjuvant resulting in the creation of anti-zona pellucida antibodies which bind to the zona pellucida of the oocyte and block sperm attachment to zone pellucida receptors - and the nature and fate of the product's metabolites, do not suggest that the product has the potential to be toxic or pathogenic. This is further supported by information that shows that once the product is ingested, it is broken down to amino acids and simple carbohydrates which do not cause an immune response and are biologically inactive. Additionally, PZP and the adjuvant antigens are not stored in body tissues.

a. Toxicological End Points

No toxicological end points were established.

b. Dietary Exposure

The Agency has determined that neither a tolerance nor a tolerance exemption is necessary for this active ingredient at this time. In the past, horse slaughter plants existed in the United States and some horse meat was used for animal feed and human food (though virtually all meat was exported). EPA does not believe there are any horse slaughter plants currently operating in the United States. Because of this and the BLM assertion that no horses or burros will be sold to slaughter houses, the Agency is confident that treated animals will not be used as food or feed.

The Agency has also determined that this use of PZP would not result in residues in treated animals. Once the contraceptive is injected into the animal, both components of the contraceptive are detected by the humoral immune system and are broken down into resulting products that bear no resemblance to the original contraceptive and are excreted and eliminated from the body in forms that cannot be distinguished from other metabolic products, such as CO_2, water, lactic acid, and urea. Likewise, the antibodies that are produced in response to ZonaStat-H injection are broken down into their component amino acids, and recycled into other body proteins or metabolized and excreted as urea, CO_2, and water.

c. Occupational Risk

Applicators could potentially be exposed to ZonaStat-H by dermal or ocular routes while loading a syringe or by accidental self-injection. There are no occupational concerns as a result of potential dermal or ocular exposure because PZP is a weak antigen and is unlikely to be absorbed intact for the same reason described in the Dietary Exposure section.

Handler Exposure and Risk: Accidental self-injection could result in infertility in females, with no reproductive effect on males. Though public literature indicates that the contraceptive effects of PZP treatment administered annually for up to 5 years to horses are reversible (Kirkpatrick and Turner, 2002), there are no data available for humans exposed to PZP. There is evidence that annual treatment of horses for longer than 7 years results in irreversible infertility, but it is unlikely that accidental self-injection would occur routinely. The ZonaStat-H label includes the statements: "If pregnant, take precaution when preparing, loading and recovering darts to not self-inject. Accidental injection may cause infertility in women."

A physical injury could occur as a result of self-injection, especially if there was tissue trauma from a dart gun. The likelihood of accidental self-injection will be minimized because the product is classified as a Restricted Use Pesticide used only by trained certified applicators or persons under their direct supervision (see Section IV). Applicators are required to wear latex or vinyl examination gloves when handling the product and during all operations in which accidental dermal exposure could occur, including washing of mixing syringes.

Occupational Post-Application Exposure and Risk: There is the possibility of post application exposure through contact with an undischarged dart. Dart recovery data of 329 different horses treated with PZP are available for 3 sites – Assateague Island National Seashore, MD where 1,185 darts were fired in which 1,115 were recovered from 1994-2007; Cape Lookout National Seashore, NC, fired 313 darts and recovered 301 for the years 2001-2007; and 146 darts were fired at Little Book Cliffs Wild Horse Range, CO, with 140 recovered for the years 2003-2007.

While individuals using the dart guns reportedly made every effort to retrieve darts whether they struck the target or not, approximately 5% of the darts were not recovered (as reported above). Some of the darts that missed the horse would have discharged upon striking the ground or surrounding brush resulting in degradation of the glycoprotein into the environment. Therefore, it is believed that only a small amount of unrecovered darts would have retained their contents. Even if an unretrieved dart still retained its contents, exposure to humans or the environment is unlikely because a significant impact is required with enough velocity to result in discharge of the dart contents.

d. Residential Risk

A residential risk assessment was not conducted because there is no residential use associated with this product.

III. Environmental Risk

A summary of the environmental fate and ecological risks of PZP as assessed in the Agency document titled *"Section 3 Request for ZonaStat, a New Chemical Proposed for Use to Control Wild Horses and Burros"* dated October 4, 2010 is provided below.

Waiver requests were submitted to fulfill the required ecological effects and environmental fate guideline studies. For the reasons listed below, the waivers were granted.

Exposure to non-target organisms is not likely to occur because of the targeted nature of the application. Given the lack of potential exposures, it is unnecessary to generate most of the data generally required for outdoor uses. The Agency determined that these studies are not considered necessary to support the proposed uses of PZP for several reasons:

Due to the application route of injection to the target animal, potential exposure routes for non-target organisms resulting from labeled uses is limited. Exposure to carnivores, exposure to excreted material, and exposure to off-target darts are the potential routes of exposure identified by the Agency and these pathways are unlikely to result in potential risks to non-target organisms at levels of concern to the Agency. Since PZP is deactivated in the digestive tract and absorption from the GI tract is expected to be limited, dietary exposure to ZonaStat is not expected to result in adverse effects at levels of concern to the Agency. Additionally, the short half-life in treated mammals suggests that the potential for secondary exposure to carnivores or scavengers is limited. PZP is not excreted intact from treated animals; it breaks down into amino acids and simple carbohydrates. Off-target, intact darts result in exposure to PZP if the non target contacts the dart with enough impact that the contents are injected. However, it is important to note that PZP has a short shelf life of only 24 hours when removed from frozen storage.

There is very limited potential for water contamination through the use of this product as the product is not expected to be excreted intact from treated animals. The only exposure to aquatic ecosystems would be through darts that missed their target. A dart contains 100 µg of active ingredient and even if the contents of a dart were to enter a small, 20,000,000 liter pond the resulting concentration would be 0.005 ng/L.

Potential exposures to non-target animals are not expected to result in any significant risk concerns to terrestrial or aquatic organisms from the proposed use of PZP.

IV. Regulatory Decision

Consistent with the requirements of FIFRA section 3(c)(5), EPA is unconditionally registering the product ZonaStat-H, containing porcine zona pellucida to be used as a contraceptive for wild horses and burros. Pursuant to the provisions of section 3(c)(4) of FIFRA, the Agency published a Notice of Receipt (NOR) of the registration application in the *Federal Register* on January 27, 2010 (Docket No. EPA-HQ-OPP-2009-0800). One anonymous comment was submitted in response to the NOR, it did not present any new information or data, but implied that equines on BLM managed lands treated with PZP could end up in the food chain and questioned EPA's jurisdictional regulatory oversight on contraceptive vaccines. The BLM has asserted on their website
http://www.blm.gov/wo/st/en/prog/wild_horse_and_burro/wh_b_information_center/Fact_Sheet.html
that "The BLM has not been selling any wild horses or burros to slaughterhouses or to "killer buyers." During the comment period on the proposed registration decision for ZonaStat-H, one comment was submitted that expressed support for its registration. The commenter is developing another contraceptive for horses. In addition to supporting the registration, he also offered suggestions and critiques regarding the Training Manual. These comments will be passed on to the HSUS for their consideration.

A. Data Requirements

No additional data is required to support the proposed registration.

B. Labeling Requirements

The following requirements have been imposed:

- Restricted-Use Pesticide classification limiting application to Department of Interior, and all its designated agents (i.e., National Park Service, Bureau of Land Management, U.S. Fish &Wildlife Service); State departments of agriculture/livestock and wildlife, and their designated agents; Federally recognized Indian tribes, and their designated agents; Department of Defense and its designated agents; Public and private wild horse sanctuaries and reserves; Humane Society of the United States designated agents; USDA and all its designated agents (i.e., U.S. Forest Service, Animal and Plant Health Inspection Service).

- Use limited to only two animals: Wild and feral horses (*Eqqus caballus*) and feral burros (*Eqqus asinus*).

- Label statement restricting the application of ZonaStat-H to horses or burros that will not be used as food or feed.

- Personal Protective Equipment requirements include: long sleeved shirt and long pants, gloves and shoes plus socks to mitigate occupational exposure.

- Advisory statement for female applicators: "If pregnant, take precaution when preparing, loading and recovering darts to not self-inject. Accidental injection may cause infertility in women."

United States
Environmental Protection Agency
Office of Chemical Safety and Pollution Prevention
(7505P)

Pesticide Fact Sheet

Name of Chemical:	**Porcine Zona Pellucida (PZP)**
Reason for Issuance:	**New Chemical Nonfood Use**
Date Issued:	**January 2012**

1. Description of Chemical

Glycoprotein Complex:	ZP1 (80,000-90,000 KD), ZP2 (60,000-65,000 KD), ZP3 (55,000 KD), and ZP4 (20,000 – 25,000 KD)
Common Name:	Porcine Zona Pellucida (PZP)
EPA PC Code:	176603
Chemical Class:	Sterilant/Hormone
Registration Status:	New Chemical, nonfood use
Pesticide Type:	Mammalian Contraceptive
U.S. Technical Registrant:	Humane Society of the United States 2100 L St. NW Washington, DC 20037

2. Use Patterns and Formulations

Mode of Action:	PZP antigen is the glycoprotein layer that surrounds the oocyte and is weakly antigenic by itself. Therefore, PZP is emulsified with an adjuvant (mFCA for the primary vaccination and mFIA for booster

vaccinations) which stimulates a stronger immune response. This results in the creation of anti-zona pellucida antibodies which bind to the zona pellucida of the oocyte, alter their conformation, and block sperm attachment to the zona pellucida receptors.

Application Sites: ZonaStat-H will be used to control female wild and feral horse and burros privately or publicly owned, in areas where they have become a nuisance and are capable of doing environmental damage.

Methods of Application: The vaccine will be injected intramuscularly in hip or gluteus muscles by hand-held syringe, syringe mounted on a jabstick, or by syringe dart fired from a CO_2 or cartridge-powered projection system.

Application Rate: The application rate is 1.0 cc of PZP + adjuvant (modified Freund's complete adjuvant for the initial application, then modified Freund's incomplete adjuvant for follow-up applications). A second administration is given 2 to 4 weeks after the initial priming dose, then annually thereafter.

3. Science Findings

Available product chemistry data supporting the use of ZonaStat-H including product chemistry, toxicology, efficacy, and ecological effects and environmental fate are summarized below in Tables 1 and 1.1.

Table 1. Product Chemistry Summary

Common name	Porcine Zona Pellucida (PZP)
Color	Clear
Physical State	Active: Aqueous solution or powder EU: Thick, white aqueous emulsion
Odor	Odorless
Oxidation/Reduction Action	Denatured by acid or base, no incompatibility
pH	7.0 – 7.04
Flammability	Nonflammable (protein)
Explodability	Not explosive (protein)
Storage stability	Frozen liquid (or powder in desiccant) is viable for 2 years.
Corrosion Characteristics	No corrosive activity.

TOXICOLOGY SUMMARY

The Registrant submitted waiver requests for the acute oral, acute dermal, acute inhalation, primary eye irritation, primary dermal irritation, and dermal sensitization studies. The waiver requests were reviewed and determined to be acceptable.

Table 2. Acute Toxicity Data

GUIDELINE NO.	STUDY TYPE	MRID NO.	RESULTS	TOXICITY CATEGORY
870.1100	Acute Oral Toxicity	47859803	Waived	IV
870.1200	Acute Dermal Toxicity	47859803	Waived	III
870.1300	Acute Inhalation Toxicity	47859803	Waived	III
870.2400	Acute eye irritation	47859803	Waived	III
870.2500	Primary skin irritation	47859803	Waived	III
870.2600	Dermal sensitization	47859803	Waived	Negative

- Toxicity Category III = Precautionary Statements Required

Chronic toxicity data were not submitted. There is no human exposure from use of ZonaStat-H, therefore no toxicity endpoints were selected because of the very limited potential worker and dietary exposure.

ECOLOGICAL EFFECTS

Waivers were submitted to fulfill required ecological effects and environmental fate guideline studies for the registration of ZonaStat-H because of the limited potential for environmental releases. Since the product is labeled only for injection to target animals by hand or dart, is expected to be deactivated in the digestive tract, and has a short half-life in treated mammals, the limited potential risks to non-target organisms resulting from the proposed registration of ZonaStat-H are not expected to exceed the Agency's concern levels.

Risk to Endangered Species

The following table summarizes the conclusions of potential concerns for direct and indirect effects to federally-listed threatened and endangered species (listed species). Because the proposed uses cannot be geographically limited, all federally listed species are assumed to be potentially indirectly affected. The available data suggest that potential exposures to non-target animals is not expected to result in any significant risk concerns to terrestrial or aquatic organisms from the proposed use. However, indirect effects (potentially beneficial or negative) to Listed species could not be precluded.

Table 3. Potential Effects to Federally Listed Taxa

Listed Taxa	Direct Effects	Indirect Effects
Terrestrial and semi-aquatic plants – monocots and dicots	No	Yes
Terrestrial invertebrates	No	Yes
Birds (surrogate for terrestrial-phase amphibians and reptiles)	No	Yes
Mammals	No	Yes
Aquatic vascular plants	No	Yes
Aquatic non-vascular plants	No	Yes
Freshwater fish (surrogate for aquatic-phase amphibians)	No	Yes
Freshwater invertebrates	No	Yes
Freshwater benthic invertebrates	No	Yes
Estuarine/Marine fish	No	Yes
Estuarine/Marine crustaceans	No	Yes
Estuarine/Marine mollusks	No	Yes

EFFICACY

As ZonaStat-H does not bear claims to control pests that may pose a threat to human health, pursuant to OPPTS 810.1000(b)(2), the requirement for demonstration of efficacy is waived. In lieu of efficacy studies, the registrant provided various peer-reviewed published articles demonstrating ZonaStat's efficacy as a contraceptive for wild horses and burros.

The principle of efficacy of PZP in horses was first demonstrated by Liu et al. (1989) by inhibiting fertility in 12 of 14 captive fertile domestic and wild mares (*Eqqus caballus*), which persisted for 7 months. The researchers inoculated the mares with 4 hand injections of PZP with aluminum hydroxide gel. As the aluminum hydroxide gel was found to be only moderately effective in most of the horses, it was therefore substituted by FCA and FIA at 2-4 week intervals. A fifth booster injection was administered 6-9 months after the fourth injection. This study also demonstrated that anti-PZP antibody titers of 64% or greater were associated with effective contraception, and that a decline in contraceptive effect correlated with a decline in antibody titers.

Kirkpatrick et al. (1990) demonstrated PZP effectiveness in a study conducted at Assateague Island National Seashore (ASIS), MD in which 26 mares were remotely injected with a priming dose of 65-100 µg PZP in FCA and either one or two boosters of PZP in FIA at three-week intervals based on the determination by Liu et al. (1989) that at least two inoculations are required in horses so antibody titers are raised high enough for a minimum of 6 months. Upon the first inoculation, antigen recognition is initiated which increases antibody titers temporarily. Then, the second inoculation causes increased titers that last for several months, with each follow-up inoculation prolonging the duration of high titers (Kirkpatrick, et al. 1990).

During this study, 14 of the 26 treated mares were already pregnant upon inoculation and gave birth to healthy foals approximately 1 – 3 months after the last inoculation. By October 1998, there was only one pregnancy out of the 26 treated mares, as indicated by analysis of urinary steroids, with zero pregnancies among the 18 receiving 3 inoculations, and one pregnancy out of the 8 receiving two inoculations. The following spring, August 1989, only one of the 26 treated mares produced foals (Kirkpatrick, et al. 1990). Of the 26 treated mares, 14 were boosted again a year later with a single remotely delivered dart containing PZP in FIA. Only 1 of the 14 boosted mares was pregnant and produce a foal the following year, compared to 10 of 22 "sham-treated and untreated mares (45.5%) (Kirkpatrick, et al. 1991). Additional studies were carried out during the next 6 years which demonstrated foaling rates of 3.8% (4 foals in 105 mare-years) among PZP-treated mares compared to 46.2% in untreated mares (Kirkpatrick, et al. 1991). Zero population growth was achieved in 2 years, with an initial decline in the population becoming apparent in 8 years of inoculations and by year 11, the population declined from 175 to 135 horses, a decrease of 22.8% (Kirkpatrick and Turner 2008).

Turner et al. (1996) conducted a study at Virgin Islands National Park, St. Johns, VI (VINP) on free-roaming feral burros (*Eqqus asinus*) to assess the effectiveness of PZP as a contraceptive with results comparable to those seen in the Assateague Island studies. In this study, 16 female burros were treated with PZP contraceptive. Burros were given an initial one- or two-injection PZP treatment and, after 10 – 12 months, were given a one-injection PZP booster treatment. Initial treatment consisted of: (1) two separate injections (3 weeks apart) of a 1.0 mL emulsion, containing 65 µg PZP plus FCA (first injection) followed by a booster of FIA (n = 13); or (2) a single injection containing 130 µg PZP emulsified in FCA (n = 3). The single injection was a time-released method with release rates projected to be continuous across 4 weeks, with greatest release in weeks 1 and 4 followed by a booster shot at the end of the 4 weeks (Turner et al., 1996).

Zero of 13 females darted with a priming dose of 65 – 100 µg in FCA and a booster of 65 – 100 µg PZP in FIA produced foals in the period 12 – 24 months after treatment, while 1 of the 3 females receiving the single dose produced foals. Furthermore, 6 of 11 control females gave birth in that time period. Unlike wild and feral horses, feral burros are not seasonal breeders, and some of the burros were pregnant at the time of treatment.

The results of this study indicate the two-injection protocol was more effective than the single-injection in preventing pregnancies.

The effectiveness of the adjuvant used is an important factor in how efficacious the PZP epitope is as an immunocontraceptive (Lyda, et al. 2005). Since 1998, PZP has been used in captive free-ranging wild horses with a high degree of efficacy, utilizing Freund's Complete Adjuvant (FCA) as the adjuvant of choice for the initial inoculation and Freund's Incomplete Adjuvant (FIA) for booster inoculations. The use of FCA has resulted in 90% or greater efficacy, however two side effects can occur from its use: 1) Injection site reactions, including open abscesses and 2) false-positive tuberculosis (TB) tests in treated animals. The primary ingredient in the FCA is *Mycobacterium tuberculosis* which can cause antibodies against the TB organism. As a result of these side effects, the United States Department of Agriculture (USDA) has voiced opposition to the use of FCA.

Therefore, modified Freund's Complete Adjuvant (mFCA) has been substituted for FCA in titer trials of captive mares. These trials demonstrated no significant difference between mares hand-injected with 65-100 µg PZP in mFCA followed by a booster shot of 65-100 µg in FIA and mares treated with 65-100 µg PZP in FCA followed by a booster of 65-100 µg in FIA. Lyda et al. (2005) reported that 7 of 8 (87.5%) of mares treated with PZP and mFCA remained above the contraceptive titer threshold 10 months after treatment. The effectiveness of mFCA as an adjuvant was verified with these studies.

4. Summary of Regulatory Position and Rationale

Available data provide adequate information to support the unconditional registration of ZonaStat-H as a tool for management of nuisance feral and wild horses, and burros.

Like other animals (e.g. deer, Canada geese, etc.), horses may be pests in some situations. As a result of Federal protection, lack of natural predators, and fecundity (herd sizes can double in about four years), wild horse and burro herd populations have significantly increased, exceeding the BLM appropriate population levels of 27,200 in BLM managed lands. To help control these populations, BLM removes wild horses and burros and transfers them to private ownership or maintains them in BLM holding facilities.

With high population levels and the inability to sell or adopt out all captured wild horses and burros, the BLM has expressed that there is an explicit need to manage wild horse and burro populations because uncontrolled populations may lead to adverse environmental effects such as degradation of wildlife and native vegetation habitat. Additionally, these populations may lead to conflicts with other rangeland uses such as cattle grazing and recreation.

With these factors in mind, EPA is proposing to register ZonaStat-H and PZP for use to control wild and feral horse and burro populations. The Agency feels that ZonaStat-H will provide BLM a much needed alternative control method for wild horse and burro populations. The Agency believes that ZonaStat-H and PZP meet the standard for unconditional registration in FIFRA § 3(c)(5) including that it will not cause any unreasonable adverse effects on the environment. Therefore, the Agency proposes to grant this registration with the labeling requirements below.

5. Labeling Restrictions

To mitigate any risks, the following requirements have been imposed:

- Restricted-Use Pesticide classification limiting application to Department of Interior, and all its designated agents (i.e., National Park Service, Bureau of Land Management, U.S. Fish &Wildlife Service); State departments of agriculture/livestock and wildlife, and their designated agents; Federally recognized Indian tribes, and their designated agents; Department of Defense and its designated agents; Public and private wild horse sanctuaries and reserves; Humane Society of the United States designated agents; USDA and all its designated agents (i.e., U.S. Forest Service, Animal and Plant Health Inspection Service).

- Use limited to only two animals: Wild and feral horses (*Eqqus caballus*) and feral burros (*Eqqus asinus*).

- Label statement restricting the application of ZonaStat-H to horses or burros that will not be used as food or feed.

- Personal Protective Equipment requirements include: long sleeved shirt and long pants, gloves and shoes plus socks to mitigate occupational exposure.

- A warning that pregnant women must not be involved in handling or injecting ZonaStat-H and that all women should be aware that accidental self-injection may cause infertility.

6. Data Requirements

The registrant has fulfilled all data requirements, resulting in an unconditional registration of ZonaStat-H.

7. CONTACT PERSON AT EPA

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DISCLAIMER: The information presented in this Pesticide Fact Sheet is for informational purposes only may not be used to fulfill data requirements for pesticide registration and reregistration. The information is believed to be accurate as of the date on the document.

APPENDIX I

GLOSSARY OF TERMS AND ABBREVIATIONS

ADNT	Acute delayed neurotoxicity
a.i.	Active Ingredient
aPAD	Acute Population Adjusted Dose
ARI	Aggregate Risk Index
BCF	Bioconcentration Factor
CAS	Chemical Abstracts Service
ChE	Cholinesterase
ChEI	Cholinesterase inhibition
cPAD	Chronic Population Adjusted Dose
%CT	Percent crop treated
DAT	Days after treatment
DEEM-FCID	Dietary Exposure Evaluation Model - Food Consumption Intake Database
DNA	Deoxyribonucleic acid
DNT	Developmental neurotoxicity
DIT	Developmental immunotoxicity
DWLOC	Drinking Water Level of Comparison.
EC	Emulsifiable Concentrate Formulation
EEC	Estimated Environmental Concentration. The estimated pesticide concentration in an environment, such as a terrestrial ecosystem.
EPA	U.S. Environmental Protection Agency
FQPA	Food Quality Protection Act
GLC	Gas Liquid Chromatography
GLN	Guideline Number
LC_{50}	Median Lethal Concentration. A statistically derived concentration of a substance that can be expected to cause death in 50% of test animals. It is usually expressed as the weight of substance per weight or volume of water, air or feed, e.g., mg/l, mg/kg or ppm.
LD_{50}	Median Lethal Dose. A statistically derived single dose that can be expected to cause death in 50% of the test animals when administered by the route indicated (oral, dermal, inhalation). It is expressed as a weight of substance per unit weight of animal, e.g., mg/kg.
LOAEL	Lowest Observed Adverse Effect Level
LOAEC	Lowest Observed Adverse Effect Concentration
LOC	Level of Concern
LOD	Limit of Detection
LOQ	Limit of Quantitation
mg/kg/day	Milligram Per Kilogram Per Day
mg/L	Milligrams Per Liter
MOE	Margin of Exposure
MRID	Master Record Identification (number), EPA's system of recording and tracking studies submitted
MTD	Maximum tolerated dose
NA	Not Applicable
NOEC	No Observable Effect Concentration
NOEL	No Observed Effect Level

NOAEL	No Observed Adverse Effect Level
NOAEC	No Observed Adverse Effect Concentration
NPDES	National Pollutant Discharge Elimination System
OP	Organophosphate
OPP	EPA Office of Pesticide Programs
OPPTS	EPA Office of Prevention, Pesticides and Toxic Substances
PAD	Population Adjusted Dose
PAG	Pesticide Assessment Guideline
PAM	Pesticide Analytical Method
PHED	Pesticide Handler's Exposure Data
PHI	Preharvest Interval
ppb	Parts Per Billion
PPE	Personal Protective Equipment
ppm	Parts Per Million
PRZM/EXAMS	Tier II Surface Water Computer Model
RAC	Raw Agriculture Commodity
RBC	Red Blood Cell
RED	Reregistration Eligibility Decision
REI	Restricted Entry Interval
RfD	Reference Dose
SCI-GROW	Tier I Ground Water Computer Model
SF	Safety Factor
TGAI	Technical Grade Active Ingredient
UF	Uncertainty Factor
µg	micrograms
µg/L	Micrograms Per Liter
µL/g	Microliter per gram
USDA	United States Department of Agriculture
WPS	Worker Protection Standard

APPENDIX II

Citations Considered to be Part of the Data Base Supporting the Registration of Porcine Zona Pellucida.

MRID	Citation	Receipt Date
47859801	Grandy, J. (2009) ZonaStat-H (Porcine Zona Pellucida): Product Efficacy: (Wild Horses and Burros). Unpublished study prepared by The Humane Society of the United States.	September 17, 2009
	Liu, I.K.M., M. Bernoco, and M. Feldman. 1989. Contraception in mares heteroimmunized with pig zonae pellucidae. Journal of Reproduction and Fertility. 85:19-29.	September 17, 2009
	Kirkpatrick, J.F., I.K.M. Liu, and J.W. Turner, Jr. 1990. Remotely delivered immunocontraception in feral horses. Wildlife Society Bulletin. 18:326-330.	September 17, 2009
	Kirkpatrick, J.F., I.K.M. Liu, T.W. Turner, and M. Bernoco. 1991. Antigen recognition in feral mares previously immunized with porcine zonae pellucidae. Journal of Reproduction and Fertility Supplement. 44:321-325.	September 17, 2009
	Kirkpatrick, J.F. and A. Turner. 2008. Achieving population goals in a long-lived wildlife species (*Eqqus caballus*) with contraception. Wildlife Research. 35:513-519.	September 17, 2009
	Turner, J.W., I.K.M. Liu, and J.F. Kirkpatrick. 1996. Remotely delivered immunocontraception in free roaming feral burros (*Eqqus asinus*). Journal of Reproduction and Fertility. 107:31-35.	September 17, 2009
	Lyda, R.O., J.R. Hall, and J.F. Kirkpatrick. 2005. Comparison of Freund's complete and Freund's modified adjuvants used with a contraceptive vaccine in wild horses (*Eqqus caballus*). Journal of Zoo and Wildlife Medicine. 36:610-616.	September 17, 2009
47859802	Grandy, J. (2009) ZonaStat-H (Porcine Zona Pellucida): Product Identity and Composition. Unpublished study prepared by Humane Society of the United States.	September 17, 2009
47859803	Grandy, J. (2009) ZonaStat-H (Porcine Zona Pellucida): Toxicology – Acute. Unpublished study prepared by Humane Society of the United States.	September 17, 2009
47859804	Grandy, J. (2009) ZonaStat-H (Porcine Zona Pellucida): Human Exposure. Unpublished study prepared by Humane Society of the United States.	September 17, 2009
47859805	Grandy, J. (2009) ZonaStat-H (Porcine Zona Pellucida): Ecological Effects. Unpublished study prepared by Humane Society of the United States.	September 17, 2009
47859806	Grandy, J. (2009) ZonaStat-H (Porcine Zona Pellucida): Human Exposure. Unpublished study prepared by Humane Society of the United States.	September 17, 2009
47859807	Grandy, J. (2009) ZonaStat-H (Porcine Zona Pellucida): Environmental Fate. Unpublished study prepared by Humane Society of the United States.	September 17, 2009

Proposed Registration of ZonaStat-H, a Contraceptive, for Wild and Feral Horses and Burros for Population Control

Approved by: _____

Lois Rossi, Director
Registration Division

Date: 11/15/2011

11/8/2011

Proposed Registration of ZonaStat-H, a contraceptive, for wild and feral horses and burros for population control.

I. Regulatory Proposal and Introduction

The Agency is proposing to grant the first registrations of the active ingredient porcine zona pellucida (PZP) for application to wild and feral horses and burros as a contraceptive for population control. Products to be registered are a technical product for manufacturing use and an end product containing 0.01% PZP. The proposed uses for PZP are non-food uses and no tolerances are being established.

The Humane Society of the United States submitted an application for this first registration (ZonaStat-H) on September 16, 2009. The requested application use is for the control of wild and feral horse and burro populations. The Agency published a Notice of Receipt for this first registration on January 27, 2010 and received one public comment that did not present any new information or data, but implied that these Bureau of Land Management (BLM) equines (those treated with PZP) could end up in the food chain and questioned EPA's jurisdictional regulatory oversight on contraceptive vaccines. The BLM has asserted that "The BLM has not been selling any wild horses or burros to slaughterhouses or to "killer buyers." Refer to the BLM website: http://www.blm.gov/wo/st/en/prog/wild_horse_and_burro/wh_b_information_center/Fact_Sheet.html

Wild horses and burros inhabiting private and state-owned lands are the responsibility of state agencies which have to address issues involving these animals when conflicts arise. The Department of Interior (DOI), the U.S. Forest Service (USFS), and the Department of Defense (DOD) manage lands that support wild horses and burros. The Free-Roaming Wild Horse and Burro Act provides BLM the authority for managing wild horse and burro populations on BLM land. BLM also works with the USFS to manage these populations on national forests land. The National Park Service (NPS) considers wild horses and burros to be an "exotic species" or "feral livestock" and manages herds found on Park lands.

ZonaStat-H is to be injected into female wild horses and burros by one of the following three methods: hand delivery, jab-stick delivery, and remote (dart) delivery. The product is labeled as a Restricted Use Pesticide (RUP) meaning it can only be sold to and administered by certified applicators or persons under their direct supervision.

Like other animals (e.g. deer, Canada geese, etc.), horses may be pests in some situations. To the extent that expanding populations of feral horses are adversely affecting public and private lands by over-grazing, products intended to prevent, mitigate, or otherwise control horse behaviors contributing to those problems are pesticides subject to regulation under FIFRA.

II. Human Health Risk Assessment

The registrant submitted waiver requests for the toxicity studies ordinarily required for a terrestrial, non-food use pesticide. The waiver requests were granted due to lack of toxicity on the target animal; a history of safe use of the vaccine, explained further below; the mode of action and fate of the product's metabolites; the limited opportunity of exposure to non-target animals, applicators, and the public; and lack of immunotoxicity as shown in the published scientific literature.

Under the sponsorship of the National Park Service (NPS), testing of ZonaStat-H began in 1988 on wild horses at Assateague Island National Seashore (ASIS) and it has subsequently been tested on white-tailed deer, zoo animals, African elephants, and other animals. Between 1994 and 2007, 901 female horses were darted with PZP by two applicators without any incident of report of harm to the applicators. Since 2004, ZonaStat-H has been administered to an estimated 1800 western wild horses on 47 herd management areas by researchers and BLM personnel. Also, 136 zoos have administered ZonaStat-H to captive animals with no reports of harm or adverse effects to applicators.

The basic biology and properties of ZonaStat-H, i.e., its mechanism of action - ZonaStat-H contains porcine zona pellucida antigen (the glycoprotein layer surrounding the oocyte) and an adjuvant resulting in the creation of anti-zona pellucida antibodies which bind to the zona pellucida of the oocyte and block sperm attachment to zone pellucida receptors - and the nature and fate of the product's metabolites, do not suggest that the product has the potential to be toxic or pathogenic. This is further supported by information that shows that once the product is ingested, it is broken down to amino acids and simple carbohydrates which do not cause an immune response and are biologically inactive. Additionally, PZP and the adjuvant antigens are not stored in body tissues.

a. Toxicological End Points

No toxicological end points were established as these tests were waived.

b. Dietary Exposure

The Agency has determined that neither a tolerance nor a tolerance exemption is necessary for this active ingredient at this time. In the past, horse slaughter plants existed in the United States and some horse meat was used for animal feed and human food (though virtually all meat was exported) EPA does not believe there are any horse slaughter plants currently operating in the United States. Because of this and the BLM assertion that no horses or burros will be sold to slaughter houses, the Agency is confident that treated animals will not be used as food or feed.

The Agency has also determined that this use of PZP would not result in residues in treated animals. Once the contraceptive is injected into the animal, both components of the

contraceptive are detected by the humoral immune system and are broken down into resulting products that bear no resemblance to the original contraceptive and are excreted and eliminated from the body in forms that cannot be distinguished from other metabolic products, such as CO_2, water, lactic acid, and urea. Likewise, the antibodies that are produced in response to ZonaStat-H injection are broken down into their component amino acids, and recycled into other body proteins or metabolized and excreted as urea, CO_2, and water.

Further, even if PZP were to be consumed, there would be no dietary risk. In a study in which rabbits were fed PZP, the analysis showed that rabbits did not develop circulating anti-PZP IgG antibodies. The number of embryos in treated rabbits was not affected when compared to controls (Barber and Fayrer-Hosken, 2000).

c. Occupational Risk

Applicators could potentially be exposed to ZonaStat-H by dermal or ocular routes while loading a syringe or by accidental self-injection. There are no occupational concerns as a result of potential dermal or ocular exposure because PZP is a weak antigen and is unlikely to be absorbed intact for the same reason described in the Dietary Exposure section.

Handler Exposure and Risk: Accidental self-injection could result in infertility in females, with no reproductive effect on males. Though public literature indicates that the contraceptive effects of PZP treatment administered annually for up to 5 years to horses are reversible (Kirkpatrick and Turner, 2002), there are no data available for humans exposed to PZP. There is evidence that annual treatment of horses for longer than 7 years results in irreversible infertility, but it is unlikely that accidental self-injection would occur routinely. The ZonaStat-H label includes the statements: "If pregnant, take precaution when preparing, loading and recovering darts to not self-inject. Accidental injection may cause infertility in women."

A physical injury could occur as a result of self-injection, especially if there was tissue trauma from a dart gun. The likelihood of accidental self-injection will be minimized because the product is classified as a Restricted Use Pesticide used only by trained certified applicators or persons under their direct supervision (see Section IV). Applicators are required to wear latex or vinyl examination gloves when handling the product and during all operations in which accidental dermal exposure could occur, including washing of mixing syringes.

Occupational Post-Application Exposure and Risk: There is the possibility of post application exposure through contact with an undischarged dart. Dart recovery data of 329 different horses treated with PZP are available for 3 sites – Assateague Island National Seashore, MD where 1,185 darts were fired in which 1,115 were recovered from 1994-2007; Cape Lookout National Seashore, NC, fired 313 darts and recovered 301 for the years 2001-2007; and 146 darts were fired at Little Book Cliffs Wild Horse Range, CO, with 140 recovered for the years 2003-2007.

While individuals using the dart guns reportedly made every effort to retrieve darts whether they struck the target or not, approximately 5% of the darts were not recovered (as reported above). Some of the darts that missed the horse would have discharged upon striking

the ground or surrounding brush resulting in degradation of the glycoprotein into the environment. Therefore, it is believed that only a small amount of unrecovered darts would have retained their contents. Even if an unretrieved dart still retained its contents, exposure to humans or the environment is unlikely because a significant impact is required with enough velocity to result in discharge of the dart contents.

b. Residential Risk

A residential risk assessment was not conducted because there is no residential use associated with this product.

III. Environmental Risk Assessment

a. Environmental Fate and Exposure and Non-Target Organisms

Waiver requests were submitted to fulfill the required ecological effects and environmental fate guideline studies. For the reasons listed below, the waivers were granted.

Exposure to non-target organisms is not likely to occur because of the targeted nature of the application. Given the lack of potential exposures, it is unnecessary to generate most of the data generally required for outdoor uses. The Agency determined that these studies are not considered necessary to support the proposed uses of PZP for several reasons:

Due to the application route of injection to the target animal, potential exposure routes for non-target organisms resulting from labeled uses is limited. Exposure to carnivores, exposure to excreted material, and exposure to off-target darts are the potential routes of exposure identified by the Agency and these pathways are unlikely to result in potential risks to non-target organisms at levels of concern to the Agency. Since PZP is deactivated in the digestive tract and absorption from the GI tract is expected to be limited, dietary exposure to ZonaStat is not expected to result in adverse effects at levels of concern to the Agency. Additionally, the short half-life in treated mammals suggests that the potential for secondary exposure to carnivores or scavengers is limited. PZP is not excreted intact from treated animals; it breaks down into amino acids and simple carbohydrates. Off-target, intact darts result in exposure to PZP if the non target contacts the dart with enough impact that the contents are injected. However, it is important to note that PZP has a short shelf life of only 24 hours when removed from frozen storage.

There is very limited potential for water contamination through the use of this product as the product is not expected to be excreted intact from treated animals. The only exposure to aquatic ecosystems would be through darts that missed their target. A dart contains 100 μg of active ingredient and even if the contents of a dart were to enter a small, 20,000,000 liter pond the resulting concentration would be 0.005 ng/L.

Potential exposures to non-target animals are not expected to result in any significant risk concerns to terrestrial or aquatic organisms from the proposed use of PZP.

IV. EPA's Proposed Registration Decision

As a result of Federal protection, lack of natural predators, and fecundity (herd sizes can double in about four years), wild horse and burro herd populations have significantly increased, exceeding the BLM appropriate population levels of 27,200 in BLM managed lands. To help control these populations, BLM removes wild horses and burros and transfers them to private ownership or maintains them in BLM holding facilities – 74,000 were removed from BLM managed land, with only 46,400 adopted or sold in 2001, and in 2008 BLM was holding over 30,000 wild horses and burros. (Donaldson and Kiely, 2009)

With high population levels and the inability to sell or adopt out all captured wild horses and burros, the BLM has expressed that there is an explicit need to manage wild horse and burro populations because uncontrolled populations may lead to adverse environmental effects such as degradation of wildlife and native vegetation habitat. Additionally, these populations may lead to conflicts with other rangeland uses such as cattle grazing and recreation.

With these factors in mind, EPA is proposing to register ZonaStat-H and PZP for use to control wild and feral horse and burro populations. The Agency feels that ZonaStat-H will provide BLM a much needed alternative control method for wild horse and burro populations. The Agency believes that ZonaStat-H and PZP meet the standard for registration in FIFRA § 3(c)(5) including that it will not cause any unreasonable adverse effects on the environment. Therefore, the Agency proposes to grant these registrations with the labeling requirements below.

Labeling Restrictions

The following label restrictions are required:

- Restricted-Use classification limiting retail sale and use to the following persons:
    - Certified Applicators or persons under their direct supervision of the following organizations and their designated wildlife management personnel and only for those uses covered by the Certified Applicators certification:
    - National Park Service, Bureau of Land Management, U.S. Fish & Wildlife Service and Federal Land Management Agency
    - State departments of agriculture/livestock and wildlife, and their designated agents
    - Federally recognized Indian tribes, and their designated agents
    - Department of Defense and its designated agents
    - Public and private wild horse sanctuaries and reserves
    - Humane Society of the United States and designated agents

- Use limited to only two animals: Wild and feral horses (*Eqqus caballus*) and feral burros (*Eqqus asinus*).

- Label statement restricting the application of ZonaStat-H to horses or burros that will not be used as food or feed.

- Personal Protective Equipment requirements include: long sleeved shirt and long pants, gloves and shoes plus socks to mitigate occupational exposure.

- If pregnant, women are advised to take precaution when preparing, loading and recovering darts so as to not self-inject.

References:

Anderson, B., N. Andrews, E. Odenkirchen, and S. Sankula (2010). "Section 3 Request for Zonastat, a New Chemical Proposed for Use to Control Wild Horses and Burros." Environmental Fate and Effects Division, U.S. Environmental Protection Agency.

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GAO. 2008. Report to the Chairman, Committee on Natural Resources, House of Representatives, Effective Long-Term Options Needed to Manage Unadoptable Wild Horses,

Bureau of Land Management, October 2008. U.S. Government Accountability Office. Washington DC. http://www.gao.gov/new.items/d0977.pdf

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Recommendation of Division Directors
Negotiated Due Dates

Decision #: 420440	**Registration #:** 86833-R	**Petition #:** N/A

☑ *See page 2 for additional registration entries*

Chemical Name: Porcine Zona Pellucida

Fee Category: R110	**PRIA Decision Time Frame:** 20 months	
Submitted by: Jennifer Gaines	**Branch:** OCSPP/OPP/RD	**Date:** 11/29/2011

Company: Humane Society of the United States (HSUS)

Original PRIA Due Date: 05/29/2011	**Proposed New PRIA Due Date:** 01/30/2012

Previous Negotiated Due Dates: 08/31/2011 11/30/2011

Is the "Fix" in-house? ☑ Yes ☐ No ☐ n/a	**If not, date "Fix" expected:**

Negotiated Due Date Reason:

Additional Data Required	☐ Product Chemistry	☐ Toxicology	☐ Acute Tox	☐ Environmental	
	☐ Efficacy	☐ Ecological	☐ Residue	☐ Other	
Data Deficiencies	☐ Product Chemistry	☐ Acute Tox	☐ Efficacy	☐ Residue	☐ Toxicology
	☐ Environmental	☐ Ecological	☐ Labeling	☐ Other	☐ Not Submitted
Late Risk Assessment	☐ Human Health	☐ Ecological			
Interim Consideration	☐ Agency Initiated	☐ Registrant Initiated			

☐ CSF	☐ Public Process	☐ Risk Issues Environmental	☐ Risk Issues Human Health
☐ Impurities Review	☐ Label	☐ Administrative-FR Notice	☑ Other – Comment Field

Summary of Deficiency Type(s): ☐ **Not Submitted (N)** ☐ **Deficiencies (D)**

Product Chemistry: ☐ **Acute Tox:** ☐ **Efficacy:** ☐ **Labeling:** ☐ **Ecological Data:** ☐ **Other (describe):** ☑

The public process was delayed due to the late submission of the information on the inert ingredients.

Describe Interactions with Company (describe when contacted and company's response including response to previous negotiated due dates):

I contacted Kathy Sanzo, the HSUS representative via email on 11/22/11 requesting the PRIA extension. She agreed to the date 1/30/2012.

"75 Day" Letter sent? ☐ **Yes, Date sent** ☑ **No and reason for none?** *Add comments on page 2*

Rationale for Proposed Due Date:

Registrant notified that this is the last negotiation? ☐ **Yes** ☐ **Not Applicable**

Approve: ☒	**Disapprove:** ☐

If disapproved, action to be taken:

OD or DOD Signature:	**Date:** 12-20-11

Decision #:	Registration #:	Petition #:

Issue(s) (describe in detail):

Comment(s):
There was no 75-day letter sent because there were no data deficiencies.

Jennifer,

Dr. Grandy agrees to another extension of the review period (hopefully the last).

Have a nice thanksgiving.

Kathy

Kathleen M. Sanzo
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From: Gaines.Jennifer@epamail.epa.gov [
mailto:Gaines.Jennifer@epamail.epa.gov]
Sent: Tuesday, November 22, 2011 11:50 AM
To: Sanzo, Kathleen M.
Subject: ZonaStat Update

Hi Kathy,

I wanted to let you know where we are with ZonaStat now. We have all of the information posted on our website for the 30-day public comment period. Since the public comment period ends on 12/16, Meredith and I realized this needs to be renegotiated one last time. We are asking for a 2 month extension making the new due date January 30, 2012. However, our goal is to have it finalized by or before Christmas.

Thanks alot,
Jennifer

Jennifer Gaines
Wildlife Biologist
U.S. Environmental Protection Agency
Insecticide-Rodenticide Branch
Registration Division (7505P)

Tel: 703 305-5967
Fax: 703 305-6309

Recommendation of Division Directors
Negotiated Due Dates

Decision #: 420440	Registration #: 86833-R	Petition #: N/A

☐ *See page 2 for additional registration entries*

Chemical Name: Porcine Zona Pellucida

Fee Category: R110		**PRIA Decision Time Frame:** 20 months	
Submitted by: Jennifer	Gaines	**Branch:** OCSPP/OPP/RD	**Date:** 08/29/2011

Company: Humane Society of the United States

Original PRIA Due Date: 05/29/2011	**Proposed New PRIA Due Date:** 11/30/2011

Previous Negotiated Due Dates: 08/31/2011

Is the "Fix" in-house? ☐ Yes ☑ No ☐ n/a	**If not, date "Fix" expected:** 11/30/2011

Negotiated Due Date Reason:

Additional Data Required	☐ Product Chemistry	☐ Toxicology	☐ Acute Tox	☐ Environmental	
	☐ Efficacy	☐ Ecological	☐ Residue	☐ Other	
Data Deficiencies	☐ Product Chemistry	☐ Acute Tox	☐ Efficacy	☐ Residue	☐ Toxicology
	☐ Environmental	☐ Ecological	☐ Labeling	☐ Other	☐ Not Submitted
Late Risk Assessment	☐ Human Health	☐ Ecological			
Interim Consideration	☐ Agency Initiated	☐ Registrant Initiated			

☑ CSF	☑ Public Process	☐ Risk Issues Environmental	☐ Risk Issues Human Health
☐ Impurities Review	☑ Label	☐ Administrative-FR Notice	☐ Other – Comment Field

Summary of Deficiency Type(s): ☐ **Not Submitted (N)** ☐ **Deficiencies (D)**

Product Chemistry: ☐ **Acute Tox:** ☐ **Efficacy:** ☐ **Labeling:** ☐ **Ecological Data:** ☐ **Other (describe):** ☐

Describe Interactions with Company (describe when contacted and company's response including response to previous negotiated due dates):

The registrant was required to submit additional information on an inert ingredient, which they have since submitted. The registrant submitted an updated CSF, label, and training manual describing how to apply ZonaStat-H which are under review. The registrant was informed of the proposed renegotiated due date and agreed to it.

"75 Day" Letter sent? ☐ **Yes, Date sent** ☑ **No and reason for none?** *Add comments on page 2*

Rationale for Proposed Due Date: The proposed date will allow enough time to review the data.

Registrant notified that this is the last negotiation? ☐ **Yes** ☑ **Not Applicable**

Approve: ☒	**Disapprove:** ☐

If disapproved, action to be taken:

OD or DOD Signature: [signature] **Date:** 8-31-11

Recommendation of Division Directors Negotiated Due Dates		
Decision#: 420440	**Registration#:** ZonaStat-H 86833-R	**Petition #:** N/A
Fee Category: R110	**PRIA Decision Time Frame:** 20 months	
Submitted by: Kimberly Nesci	**Branch:** IRB	**Date:** May 26, 2011
Company: The Humane Society of the United States		
Original Due Date: May 29, 2011	**Proposed New Due Date:** August 31, 2011	
Previous Negotiated Due Dates: none		
Is the "Fix" in-house? No	**If not, date "Fix" expected:** May 31, 2011	

Issue (describe in detail):

Label changes needed to address potential for accidental exposure.

Confirmation needed that horses treated with ZonaStat-H will not end up in channels of trade (non-food use).

CSF changes needed.

Summary of Deficiency Type(s): Not Submitted (N) or Deficiencies (D)

Product Chemistry: (D) Acute Tox: ___ Efficacy: __ Labeling: (D) Other (describe): confirmation that treated horses won't end up in channels of trade

Describe Interactions with Company (describe when contacted and company's response including response to previous negotiated due dates):

EPA has repeatedly requested revised labels and a revised CSF from the HSUS lawyer/agent since mid-April. This has since been raised directly with the HSUS.

"75-Day" Letter sent? ____ Yes _X_ No and reason for none?

This request for an extension does not pertain to a data deficiency on part of the registrant.

Rationale for Proposed Due Date:

To allow sufficient time for RD to review the new CSF; review the new label; and post a proposed decision for public comment. The proposed date was calculated from when the registrant intends to address the deficiencies: May 31, 2011. A conference call has been scheduled to help ensure the deficiencies are addressed.

Registrant notified that this is the last negotiation? ___ Yes _X_ Not Applicable

Approve: √	**Disapprove:**

If disapproved, action to be taken:

OD or DOD Signature:	**Date:** 5-27-11

EFFICACY REVIEW

PRODUCT:	ZonaStat-H
REG. NUMBER:	86833-R
DATE:	March 15, 2011
DP BARCODE:	D370425
DECISION:	420440
GLP:	N/A
CHEMICAL:	Porcine Zona Pellucida (PZP)
CHEMICAL NUMBER:	Porcine Zona Pellucida…176603
PURPOSE:	Review submitted articles to determine if product's efficacy as a contraceptive is supported.
MRID:	**47859801. Grandy, J. (2009) ZonaStat-H (Porcine Zona Pellucida): Product Efficacy: (Wild Horses and Burros). Unpublished study prepared by The Humane Society of the United States. 84 pp.**
TEAM REVIEWER:	Jennifer Gaines, Wildlife Biologist
EFFICACY REVIEWER:	Jennifer Gaines, Wildlife Biologist Jennifer Gaines 3/15/11
SECONDARY EFFICACY REVIEWER:	Venus Eagle 3/15/11

BACKGROUND:

The Humane Society of the United States (HSUS) is applying for Section 3 registration for ZonaStat-H (porcine zona pellucida) to control feral and wild horses and burros. ZonaStat-H will be registered as a Restricted Use product to be used by certified applicators only.

PRODUCT DESCRIPTION:

The active ingredient in ZonaStat-H is porcine zona pellucida (PZP), which is an immunocontraceptive vaccine that is administered to target animals via intramuscular injection in hip or gluteus muscles either by hand delivery (injection), jab-stick delivery, or remote (dart) delivery. ZonaStat-H consists of an emulsion of 2 components: (1) the antigen, a naturally occurring, chemically unmodified glycoprotein, PZP, which is extracted from pig ovaries; and (2) an adjuvant (modified Freund's Complete Adjuvant, mFCA, or Freund's Incomplete Adjuvant, FIA). PZP itself is a composite of four different acidic glycoproteins, ZP1, ZP2, ZP3, and ZP4. Approximately 70-80% of PZP is made of ZP3. mFCA consists of cell wall fragments from a non-pathogenic soil bacterium (*Mycobacterium butyricum*). *M. butyricum* fragments are suspended in a physiologically inert mineral oil and an emulsifier. FIA is identical to mFCA, but does not have the mycobacterial cell wall that is found with mFCA.

Upon injection of ZonaStat-H into a female horse or burro, anti-zona pellucida (ZP) antibodies are produced. The antibodies bind to the ZP glycoproteins that surround the egg of the injected animal, alter the glycoproteins' conformation, and block the attachment of sperm, thus preventing fertilization.

APPLICATION METHOD AND RATE

The application rate is 1.0 cc of PZP (which is already dissolved in 0.5 mL of phosphate buffered saline, PBS) + 0.5 mL adjuvant (mFCA or FIA). The antigen solution (PZP + PBS) is mixed in the field prior to use which is detailed in the Procedures section of the label as noted below:

1. Wear examination gloves while mixing and loading PZP.
2. Attach the Luer-Lok connector to one of the glass syringes (depending on method of application).
3. Place the 1.5 inch needle on the second glass syringe.
4. Draw out 0.5 cc of adjuvant.
5. Using the same syringe, draw up the 0.5 cc of PZP in the phosphate buffer saline (PBS).
6. While holding the syringe containing the vaccine carefully in order to prevent the plunger from slipping out, take off the needle and attach the syringe to the second syringe using the Luer-Lok connector.
7. Push the PZP-adjuvant mixture back and forth through the two syringes 100 times. The resulting will become thick and white.
8. Make sure that all of the emulsion is in one syringe.
9. Holding the syringe containing the emulsion very carefully, remove the other syringe, leaving the Luer-Lok on the syringe containing the emulsion.

Different materials are needed for mixing depending on the method of application. For hand delivery, the applicator would need a 3cc disposable plastic syringe with Luer-Lok, a 1.5 inch 18 g disposable sterile needle; jab-stick delivery requires a Dan-Inject® Fiskars Combi-Click Jab Stick, a 3cc disposable plastic syringe with Luer-Lok, and a monoject 1.5 inch 14 gauge disposable sterile needle; and the remote (dart) delivery method of application requires the use of a 2.0 inch 18 gauge disposable sterile needle and a 1.0 cc C-type or P-type Pneu-Dart dart with 1.25 inch or 1.5 inch barbless needle.

For hand delivery (injection) the applicator attaches a 2.0cc or 3.0cc plastic syringe to the glass syringe via the Luer-Lok, and injects the emulsion into the plastic syringe. The applicator then disconnects the glass syringe and connects an 18 gauge 1.5 in. needle to the syringe containing the emulsion.

For jab-stick delivery, the applicator injects the emulsion from the glass syringe into the plastic syringe after attaching the plastic syringe tightly into the Luer-Lok. Then the Monoject 14 gauge 1.5 in. needle is attached to the plastic syringe containing the emulsion after the glass syringe is removed. Then the plastic syringe is placed into the jab-stick.

For remote delivery (via dart), the applicator first attaches the 18 gauge, 2 in. needle to the glass syringe containing the emulsion, then injects the emulsion into the dart. Following emulsification in the field of antigen solution and adjuvant, the applicator remotely injects ZonaStat-H in the hip, gluteus, or hamstring muscles with a syringe dart fired from a CO_2 or cartridge-powered projection system.

EFFICACY OF ZONASTAT-H

As ZonaStat-H does not bear claims to control pests that may pose a threat to human health, pursuant to OPPTS 810.1000(b)(2), the requirement for demonstration of efficacy is waived. In lieu of efficacy studies, the registrant provided various peer-reviewed published articles demonstrating ZonaStat's efficacy as a contraceptive for wild horses and burros.

Reduction of free-roaming feral horse populations via contraception has been a goal of researchers since the early 1970s (Kirkpatrick et al., 1990). Various methods have been attempted leading up to the use of PZP. Initially, fertility reduction was demonstrated by using an injectable microencapsulated testosterone propionate (mTP) in stallions which resulted in an 83% decrease in foaling by mares (Kirkpatrick, et al. 1990). Delivery of mTP was done by first immobilizing the stallions and then injecting them. This method of delivery incurred high costs and stress to the animal, resulting in a remote method of delivery. Though mTP was effective in stallions, remote delivery made it difficult to deliver enough steroid to make it effective (Kirkpatrick, et al. 1990).

Another option was tried which also utilized steroid-induced fertility control, but this time the mares were the target animal. The use of ethinylestradiol-progesterone Silastic® implants showed effectiveness, but once again much stress was placed on the target animal because the method of delivery required the mare to be captured, restrained, then undergo field surgery to place the implants peritoneally (Kirkpatrick, et al. 1990). Focus then turned to immunocontraception as an alternative to steroid-induced fertility control. Efficacy had already been demonstrated for PZP by Liu et al. in 1989 by inhibiting fertility in 13 of 14 domestic and captive feral mares.

The principle of efficacy of PZP in horses was first demonstrated by Liu et al. (1989) by inhibiting fertility in 12 of 14 captive fertile domestic and wild mares (*Eqqus caballus*), which persisted for 7 months. The researchers inoculated the mares with 4 hand injections of PZP with aluminum hydroxide gel. As the aluminum hydroxide gel was found to be only moderately effective in most of the horses, it was therefore substituted by FCA and FIA at 2-4 week intervals. A fifth booster injection was administered 6-9 months after the fourth injection. This study also demonstrated that anti-PZP antibody titers of 64% or greater were associated with effective contraception, and that a decline in contraceptive effect correlated with a decline in antibody titers.

Kirkpatrick et al. (1990) demonstrated PZP effectiveness in a study conducted at Assateague Island National Seashore (ASIS), MD in which 26 mares were remotely injected with a priming dose of 65-100 µg PZP in FCA and either one or two boosters of PZP in FIA at three-week intervals based on the determination by Liu et al. (1989) that at least two inoculations are required in horses so antibody titers are raised high enough for a minimum of 6 months. Upon the first inoculation, antigen recognition is initiated which increases antibody titers temporarily. Then, the second inoculation causes increased titers that last for several months, with each follow-up inoculation prolonging the duration of high titers (Kirkpatrick, et al. 1990).

During this study, 14 of the 26 treated mares were already pregnant upon inoculation and gave birth to healthy foals approximately 1 – 3 months after the last inoculation. By October 1998, there was only one pregnancy out of the 26 treated mares, as indicated by analysis of urinary steroids, with zero pregnancies among the 18 receiving 3 inoculations, and one pregnancy out of the 8 receiving two inoculations. The following spring, August 1989, only one of the 26 treated mares produced foals. (Kirkpatrick, et al. 1990). Of the 26 treated mares, 14 were boosted again a year later with a single remotely delivered dart containing PZP in FIA. Only 1 of the 14 boosted mares was pregnant and produce a foal the following year, compared to 10 of 22 "sham-treated and untreated mares (45.5%) (Kirkpatrick, et al. 1991). Additional studies were carried out during the next 6 years which demonstrated foaling rates of 3.8% (4 foals in 105 mare-years) among PZP-treated mares compared to 46.2% in untreated mares (Kirkpatrick, et al. 1991). Zero population growth was achieved in 2 years, with an initial decline in the population becoming apparent in 8 years of inoculations and by year 11, the population declined from 175 to 135 horses, a decrease of 22.8% (Kirkpatrick and Turner 2008).

Turner et al. (1996) conducted a study at Virgin Islands National Park, St. Johns, VI (VINP) on free-roaming feral burros (*Eqqus asinus*) to assess the effectiveness of PZP as a contraceptive with results comparable to those seen in the Assateague Island studies. In this study, 16 female burros were treated with PZP contraceptive. Burros were given an initial one- or two-injection PZP treatment and, after 10 – 12 months,

were given a one-injection PZP booster treatment. Initial treatment consisted of: (1) two separate injections (3 weeks apart) of a 1.0 mL emulsion, containing 65 µg PZP plus FCA (first injection) followed by a booster of FIA (n = 13); or (2) a single injection containing 130 µg PZP emulsified in FCA (n = 3). The single injection was a time-released method with release rates projected to be continuous across 4 weeks, with greatest release in weeks 1 and 4 followed by a booster shot at the end of the 4 weeks (Turner et al., 1996).

Zero of 13 females darted with a priming dose of 65 – 100 µg in FCA and a booster of 65 – 100 µg PZP in FIA produced foals in the period 12 – 24 months after treatment, while 1 of the 3 females receiving the single dose produced foals. Furthermore, 6 of 11 control females gave birth in that time period. Unlike wild and feral horses, feral burros are not seasonal breeders, and some of the burros were pregnant at the time of treatment. The results of this study indicate the two-injection protocol was more effective than the single-injection in preventing pregnancies.

The effectiveness of the adjuvant used is an important factor in how efficacious the PZP epitope is as an immunocontraceptive (Lyda, et al. 2005). Since 1998, PZP has been used in captive free-ranging wild horses with a high degree of efficacy, utilizing Freund's Complete Adjuvant (FCA) as the adjuvant of choice for the initial inoculation and Freund's Incomplete Adjuvant (FIA) for booster inoculations. The use of FCA has resulted in 90% or greater efficacy, however two side effects can occur from its use: 1) Injection site reactions, including open abscesses and 2) false-positive tuberculosis (TB) tests in treated animals. The primary ingredient in the FCA is *Mycobacterium tuberculosis* which can cause antibodies against the TB organism. As a result of these side effects, the United States Department of Agriculture (USDA) has voiced opposition to the use of FCA.

Therefore, modified Freund's Complete Adjuvant (mFCA) has been substituted for FCA in titer trials of captive mares. These trials demonstrated no significant difference between mares hand-injected with 65-100 µg PZP in mFCA followed by a booster shot of 65-100 µg in FIA and mares treated with 65-100 µg PZP in FCA followed by a booster of 65-100 µg in FIA. Lyda et al. (2005) reported that 7 of 8 (87.5%) of mares treated with PZP and mFCA remained above the contraceptive titer threshold 10 months after treatment. The effectiveness of mFCA as an adjuvant was verified with these studies.

CONCLUSIONS

The articles submitted by the HSUS assigned MRID Number 47859801 are acceptable in that they support the efficacy of ZonaStat-H as a contraceptive for the control of wild and feral horses and burros.

Literature Cited

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C., 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

PC Code: 176603
DP Barcode: 370378
Decision #420440

MEMORANDUM

DATE: October 4, 2010

SUBJECT: Section 3 Request for Zonastat, a New Chemical Proposed for Use to Control Wild Horses and Burros

FROM: Nancy Andrews, Chief
Edward Odenkirchen, Senior Advisor
Sujatha Sankula, Lead Biologist
Brian Anderson, Biologist
Environmental Risk Branch 1/EFED (7507P)

10/04/2010 10/4/10

TO: John Hebert, Product Manager
Insecticide Rodenticide Branch
Registration Division (7505P)

1. Summary of Proposed Action:

The Humane Society of the United States is applying for Section 3 registration for ZonaStat-H (porcine Zona pellucida) to control feral and wild horses and burros. The active ingredient is porcine Zona pellucida (PZP), which is an immunocontraceptive vaccine that is administered to target animals via intramuscular injection (either by dart or by hand).

The application rate is 1.0 cc of PZP + adjuvant (modified Freund's complete adjuvant or modified Freund's incomplete adjuvant). A second administration is given 2 to 4 weeks after the initial priming dose then annually thereafter.

This memorandum discusses the data requirements and qualitatively characterizes potential risks to non-target organisms from the proposed use.

2. Status of Submitted Data

No studies have been submitted in support of this Section 3. Waivers were submitted to fulfill required ecological effects and environmental fate guideline studies. Although the proposed use could qualify as outdoor uses, there are no exposure routes to non-target plants or animals that are likely to occur (see Section 3 of this document). Given the lack of potential exposures to non-target organisms, the proposed use pattern is more consistent with an indoor use from a data requirements perspective. For indoor uses, no studies are required. However, an acute oral study in birds, acute studies in aquatic animals, and hydrolysis studies are conditionally required. These studies are not considered to be necessary for the proposed uses for the following reasons:

- **Acute oral studies in birds**: Oral exposure could occur via consumption of treated animals. However, the proposed active ingredient is a protein that is expected to be denatured after oral consumption. This is evidenced by the lack of efficacy via oral exposure (the active ingredient must be injected to target animals to be effective). Therefore, acute oral toxicity studies are not being required at this time.

- **Acute studies in aquatic animals and hydrolysis:** Potential for contamination of water is limited. The active ingredient is not expected to be excreted intact from treated animals. Therefore, exposure to aquatic systems is limited to entry of wayward darts. A dart contains 100 ug of active ingredient (MRID 47859805). Therefore, even if the contents of a dart were to enter a pond the size of EPA's standard ecological water body of 20,000,000 L, the resulting concentration would be 0.005 ng/L.

Due to the limited possibility of exposure occurring to non-target aquatic or terrestrial animals, no data are being required at this time for the proposed use.

3. Potential Exposure and Risk to Non-Target Organisms

Given that the proposed administration route is by injection (either dart or hand) to the target animal, potential exposure routes for non-target organisms resulting from labeled uses is somewhat limited. Potential exposure pathways to non-target organisms could include secondary exposure to carnivores, exposure to excreted material, and exposure from wayward darts (accidental exposure). All of these exposure pathways, however, are considered unlikely to result in potential risks to non-target organisms at levels of concern to the Agency as described below.

Dietary exposure to Zonastat is not expected to result in adverse effects at levels of concern to the Agency because it is a protein that is anticipated to be deactivated in the digestive tract, and absorption from the GI tract is expected to be limited. Also, excretion data indicates that the short half-life in treated mammals suggests that the potential for secondary exposure is limited.

Potential exposure to aquatic environments is expected to be limited to accidental exposures. However, as previously discussed, even if a wayward dart were to enter a water body and all of the Zonastat within the dart were to enter a water body, the resulting concentration would be negligible ($5x10^{-9}$ mg/L). Because the active ingredient is a protein, it would be expected to be rapidly degraded in natural waters.

4. Threatened and Endangered Species Concern

4.1. Action Area

For listed species assessment purposes, the action area is considered to be the area affected directly or indirectly by the Federal action and not merely the immediate area involved in the action. At the initial screening-level, the risk assessment considers broadly described taxonomic groups and so conservatively assumes that listed species within those broad groups are co-located with the pesticide treatment area. This means that terrestrial plants and wildlife are assumed to be located on or adjacent to the treated site and aquatic organisms are assumed to be located in a surface water body adjacent to the treated site. The assessment also assumes that listed species are located within an assumed area, which has the relatively highest potential exposure to the pesticide, and that exposures are likely to decrease with distance from the treatment area.

If the assumptions associated with the screening-level action area result in RQs that are below the listed species LOCs, a "no effect" determination conclusion is made with respect to listed species in that taxa, and no further refinement of the action area is necessary. Furthermore, RQs below the listed species LOCs for a given taxonomic group indicate no concern for indirect effects upon listed species that depend upon the taxonomic group covered by the RQ as a resource. However, in situations where the screening assumptions lead to RQs in excess of the listed species LOCs for a given taxonomic group, a potential for a "may affect" conclusion exists and may be associated with direct effects on listed species belonging to that taxonomic group or may extend to indirect effects upon listed species that depend upon that taxonomic group as a resource. In such cases, additional information on the biology of listed species, the locations of these species, and the locations of use sites could be considered to determine the extent to which screening assumptions regarding an action area apply to a particular listed organism. These subsequent refinement steps could consider how this information would impact the action area for a particular listed organism and may potentially include areas of exposure that are downwind and downstream of the pesticide use site.

For this assessment, RQs were not calculated. However, use of Zonastat could result in the following environmental effects:

1. Reduction in wild horses/burros;
2. Increased risk of death and morbidity resulting from allergic responses.

These possible environmental effects could affect listed species by the following:

- Reduction in wild horse populations could constitute an avenue for effects on listed species by reducing prey base of listed species (negative effect);
- Reduction in wild horse populations could result in habitat responses that may positively affect listed species due to reductions in overpopulation;
- Increased presence of moribund animals that may occur via allergic responses could result in increased carrion availability (i.e. food for condors, positive effect);

4.2. Co-Location Analysis

To determine whether the proposed uses are geographically associated with known locations of listed species, a screening-level search of the LOCATES (version 2.10.3) database is typically conducted. The database compared county-level location data for listed species with county-level crop production data (as available in the 2002 agricultural census) to identify any coarse overlaps of listed species with the proposed labeled uses of Zonastat. Listed species are those that are currently on the Federal list of endangered and threatened wildlife and plants. However, for the current proposed registration for use of Zonastat on wild horses and burros, there are no geographical limitations included on the labels, and geographical limitations regarding where Zonastat may be used are uncertain. Therefore, it is assumed that Zonastat may be used in any county in the United States for the screening level analysis. For this reason, it is assumed that every federally listed species may be affected by the proposed uses of Zonastat, and a LOCATES analysis was not conducted.

The LOCATES database identifies those U.S. counties that include non-crop and turf areas and that have federally-listed endangered or threatened species that may be directly or indirectly affected. The list of affected species derived from LOCATES was not included in this assessment because the uses cover most of the United States and the direct and indirect effects includes most species. With additional refinement by exploring more detailed use patterns and species biology (e.g., geographic location, specific feeding habits, time of year likely to utilize crop fields), some species listed may be determined to be not likely to be affected.

4.3. Taxonomic Groups Potentially at Risk

A summary of the risk conclusions and direct and indirect effects determinations is presented in **Table 1**. Because the proposed uses cannot be geographically limited, all federally listed species are assumed to be potentially indirectly affected.

Table 1. Potential Listed Species Risks Associated with Direct or Indirect Effects Due to the Proposed Use of Zonastat[1]

Listed Taxonomy	Direct Effects	Indirect Effects
Terrestrial and semi-aquatic plants – monocots and dicots	No	Yes
Terrestrial invertebrates	No	Yes
Birds (surrogate for terrestrial-phase amphibians and reptiles)	No	Yes
Mammals	No	Yes

Table 1. Potential Listed Species Risks Associated with Direct or Indirect Effects Due to the Proposed Use of Zonastat[1]		
Listed Taxonomy	**Direct Effects**	**Indirect Effects**
Aquatic vascular plants	No	Yes
Aquatic non-vascular plants	No	Yes
Freshwater fish (surrogate for aquatic-phase amphibians)	No	Yes
Freshwater Invertebrates	No	Yes
Freshwater Benthic Invertebrates	No	Yes
Estuarine/Marine Fish	No	Yes
Estuarine/Marine Crustaceans	No	Yes
Estuarine/Marine Mollusks	No	Yes
1. Effect on listed species may be negative or beneficial		

5. Conclusions

The available data suggest that potential exposures to non-target animals is not expected to result in any significant risk concerns to terrestrial or aquatic organisms from the proposed use. However, indirect effects (potentially beneficial or negative) to Listed species could not be precluded.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

MEMORANDUM

Date: July 8, 2010

SUBJECT: ZONASTAT-H. Immunocontraceptive Vaccine for Limiting the Population of Wild and Feral Horses and Burros.

PC Code: 176603
Decision No.: 420440
Petition No.: N/A
Risk Assessment Type: Non-food use
TXR No.: N/A
MRID No.: N/A

DP Barcode: D370377
Registration No.: N/A
Regulatory Action: Section 3
Case No.: N/A
CAS No.: N/A
40 CFR:N/A

Ver.Apr. 2010

FROM: Kit Farwell, D.V.M.
Risk Assessment Branch VII
Health Effects Division (7509P)
Office of Pesticide Programs

THROUGH: Michael Metzger, Branch Chief
Risk Assessment Branch VII
Health Effects Division (7509P)
Office of Pesticide Programs

TO: Jennifer Gaines, Product Manager
Registration Division, IRB (7505P)
Office of Pesticide Programs

I. CONCLUSIONS

HED has no objections to the Section 3 registration of ZonaStat-H. There are no occupational or postapplication concerns for human health risk because of the very limited potential for human exposure. It is recommended that ZonaStat-H be administered by hand injection when possible because of the slightly increased occurrence of abscesses when using a dart rifle.

II. BACKGROUND

The Humane Society of the United States has applied for a Section 3 registration for ZonaStat-H. ZonaStat-H is an injectable immunocontraceptive vaccine and is to be used by certified applicators only. The registrant intends its use on wild horses and burros.

III. DISCUSSION

Ingredients: ZonaStat-H contains porcine zona pellucida antigen (the glycoprotein layer surrounding the oocyte) and an adjuvant. The adjuvant is used to stimulate a more robust immune response because porcine zona pellucida (PZP) is weakly antigenic by itself. Modified Freund's Complete Adjuvant (mFCA) is used for primary vaccination and Freund's Incomplete Adjuvant (FIA) is used for booster vaccinations.

Ovaries are collected from freshly slaughtered pigs at USDA inspected slaughterhouses and frozen. Screening for bacterial pathogens is conducted for each batch. The oocytes are isolated and zona pellucidae are collected, diluted, frozen, and protein concentration is determined by electrophoresis.

Mode of Action: Vaccination causes the production of anti-zona pellucida antibodies, which bind to the zona pellucida of the oocyte and block sperm attachment to zona pellucida receptors.

Treatment of Horses and Burros: The antigen and adjuvant are mixed shortly before injection using two glass syringes connected by a luer lock. Intramuscular injection is made into the hip or gluteus muscles. The registrant proposes delivery by hand-held syringe, jabstick, or by a syringe dart fired by a blow-pipe, CO_2-powered gun, or .22 caliber dart rifle.

The priming dose of PZP with mFCA is followed in 2 – 4 weeks by a booster of PZP with FIA. Annual boosters are of PZP with FIA. Contraceptive efficacy was found to be greatest when the booster is delivered 1 – 3 months before the beginning of the breeding season. A single priming dose is also effective at a reduced level when delivered 1 – 3 months before the breeding season.

Guideline Testing: The registrant submitted waiver requests for the subchronic, developmental, reproductive, genotoxicity, neurotoxicity, and immuntoxicity studies ordinarily required of a terrestrial, non-food use pesticide. There are currently no guideline requirements specific for testing contraceptives in wildlife.

The waiver request are granted based on the lack of toxicity to the target animal; history of safe use of the vaccine; the mode of action and fate of the product's metabolites; the limited opportunity of exposure to non-target animals, applicators, and the public; and lack of immunotoxicity as shown in the published scientific literature.

There are numerous published journal articles on the use of PZP antigen in horses in the package provided by the registrant as well as found in a literature search. These included reports from Assateague Island National Seashore, Maryland; Shackleford Banks, North Carolina; Elko and

Ely Districts of Nevada Bureau of Land Management; Little Book Cliffs Wild Horse Range, Colorado; McCullough Peaks Herd Management Area, Wyoming; Pryor Mountain Wild Horse Range, Wyoming; an un-named Northern California wild horse sanctuary; and Virgin Islands National Park, St. John (burros).

The articles provide an extensive literature on efficacy, safety in target animals, mode of action, and describe management options for use of the vaccine. The articles were generally well written and described the methods and limitations of making observations on wild, free-roaming horses. However, the articles were not intended for regulatory purposes and sometimes different articles emphasized different aspects, such as safety or efficacy, in the same herds over overlapping time periods which sometimes made interpretation difficult for this risk assessment.

As noted earlier, 2 different adjuvants are used with PZP in ZonaStat-H in the proposed registration. The adjuvant used in the primary vaccination is Modified Freund's Complete Adjuvant (**mFCA**) and the adjuvant used in subsequent boosters is Freund's Incomplete Adjuvant (**FIA**).

The early studies used Freund's Complete Adjuvant (**FCA**) in the initial injection instead of mFCA. Beginning in 2002, mFCA was substituted for FCA for the initial injection, as is currently proposed for registration. Because generally similar results were reported regardless of which adjuvant was used for initial vaccination, and because there are so much data for the earlier studies, this risk assessment reports results from studies using both priming adjuvants.

Dietary Exposure: The potential for human dietary exposure through consumption of horse meat is assessed because there used to be horse slaughter plants in this country. It is concluded that there would be little likelihood of human systemic exposure to PZP through dietary exposure because PZP is a glycoprotein which is too large to pass through membranes of the digestive tract intact. Digestion into component amino acids and simple sugars in the stomach and small intestine would occur before absorption. Even if intact PZP were somehow to be absorbed, it is weakly antigenic and requires an adjuvant to stimulate an immune response when injected.

This was confirmed in a study in which rabbits were fed PZP: 4 NZW rabbits per group were treated orally with 400 μg PZP + S-TDCM adjuvant and 4 were treated with adjuvant alone. This compares to 100 μg PZP per dose of ZonaStat-H. ELISA analysis showed that rabbits did not develop circulating anti-PZP IgG antibodies when tested at dilutions of 1:10 to 1:1000. The number of embryos and stage of embryos in treated animals was not affected when compared to controls (Barber and Fayrer-Hosken, 2000).

Occupational Exposure: Applicators could potentially be exposed to ZonaStat-H by dermal or ocular routes while loading a syringe or by accidental self-injection. There are few concerns for dermal or ocular exposure because PZP a weak antigen and is unlikely to be absorbed intact for the same reasons as described in the Dietary section above.

Accidental self-injection could result in the same effects in humans as occur in horses, *i.e.* infertility. A physical injury could also occur as a result of self-injection, especially if there was tissue trauma from a dart gun. The likelihood of accidental self-injection will be minimized

because the product is intended for use as a restricted pesticide used only by certified applicators. According to the registrant, ZonaStat-H has been administered to approximately 2,700 horses by dart, hand injection, or jab stick without reported injuries by the applicators (MRID 47859806). A summary of training requirements for applicators is shown in the Appendix.

Postapplication Exposure: There is the possibility of postapplication exposure through contact with a dart which had not discharged. Dart recovery records are available for 3 sites (MRID 47859806). At Assateague Island National Seashore, Maryland, for the years 1994 – 2007, there were 1,185 darts fired of which 1,115 were recovered. At Cape Lookout National Seashore, North Carolina, there were 313 darts fired and 301 recovered for the years 2001 – 2007. At Little Book Cliffs Wild Horse Range, Colorado, there were 146 darts fired and 140 recovered for the years 2003 – 2007.

Individuals using the dart guns reportedly made every effort to retrieve darts whether they struck the target or not. But as reported above, approximately 5% of darts were not recovered when used in different types of terrain: beaches/dunes/forest/marsh in Maryland and North Carolina and canyon/plateau/ forest/grassland in Colorado. It was not reported how many of the darts struck their target and had discharged the vaccine, but it is believed likely that the majority of darts struck their target and had discharged the contents appropriately. Of the darts which missed the horse, some would have discharged the contents upon striking brush or the ground. Degradation of the glycoprotein in the environment would then occur with no concerns for exposure by this scenario.

It is therefore believed that only a small percentage of unrecovered darts would have retained the contents. Human or environmental exposure to vaccine in these darts is unlikely because discharge requires a significant impact with sufficient velocity to set off the charge releasing the contents. According to the registrant, "Striking, stepping on, jiggling, biting, or otherwise casually moving or contacting the dart will not discharge or release the contents of the dart (MRID 47859806).

Safety to Horses: The articles evaluated safety in horses as related to injection site reactions, longevity and body condition, developmental/reproductive effects, and behavioral effects.

Longevity and body condition: Treatment of mares at Assateague Island National Seashore was associated with a greatly increased lifespan. In the study group, there were 42 untreated mares which lived an average of 6 years, 11 mares treated < 3 years lived an average of 10 years, and 19 mares treated for ≥ 3 years lived an average of 19 years (Kirkpatrick and Turner, 2007).

The greatly increased lifespan in treated mares is believed due to the reduced physiological stresses of gestation and lactation. Body condition scores for mares were consistently lower for lactating mares than non-lactating mares (Turner and Kirkpatrick, 2002 and Ransom, et al, 2010).

Injection site reactions: Nodules (~25 mm in diameter) were reported commonly after injection of either PZP/mFCA or PZP/FIA after darting. Abscesses were relatively rare, but were slightly

more common in horses that were darted than in horses that were hand injected. Also reported were swelling and stiffness.

There were opportunities for long-term observations of the horses on Assateague Island, some of which were acclimated to humans. There may have been fewer opportunities for long-term observations of free-roaming horses in the western states, although these horses were sometimes kept in a holding pen after injection for a long enough time for close observation for lesions. The authors of the various studies did a generally good job of describing limitations of the studies and opportunities for observation.

At Assateague Island, there were 3 abscesses after 381 treatments by dart gun or jab stick (0.7%), 2 of which occurred after use of FCA and 1 after use of FIA (Kirkpatrick, et al, 1990 and Lyda, et al, 2005). In a study in Nevada, no abscesses were observed after hand injection of 60 wild mares using PZP/FCA and PZP/FIA (Turner, et al, 1997). Also reported for Nevada mares (Turner, et al, 2001), no abscesses were observed after 155 mares received 2 injections hand injections each (PZP/FCA and PZP/FIA, some also received Carbopol® adjuvant).

Another study in Nevada compared injection site reactions with two adjuvants using hand injection. The initial injection was with PZP/FCA for 7 mares and was PZP/mFCA for 8 mares. The booster for both groups was PZP/FIA. The only injection site reaction was an abscess which followed booster injection with FIA and healed without incident (Lyda, et al, 2005).

One article compared type of injection site reaction with methods of injections (hand injection, CO_2 blowgun, or .22 caliber dart rifle) and adjuvant (FCA, mFCA, FIA). Two herds in Wyoming and one in Colorado were assessed (Roelle and Ransom, 2009). Reactions following hand injection were rare: out of 100 hand injections there was 1 nodule and 2 observations of swelling. In the 2 herds that were darted, 25% of the horses had nodules (both herds), 11% and 33% had swelling, 1% and 12% had stiffness, and 1% and 6% had abscesses. Nodules were the most common reaction and sometimes persisted for a year, but did not cause noticeable change in range of movement or locomotion. Abscesses were too rare for analysis of covariates; and 4 of the 8 observed abscesses occurred in a single mare. There was no relationship between type of adjuvant and injection site reactions, suggesting that reactions are more associated with trauma from dart delivery rather than adjuvant alone.

Behavioral effects: The social behavior of horses treated with PZP was evaluated in several studies. There were only minor effects noted, as described below.

The behavior of 43 mares on Assateague Island National Seashore was observed for 3 months during the 1997 breeding season. Mares were either being currently treated with PZP or had previously been treated with PZP; untreated controls were not available. There were no significant differences between currently treated and previously treated Assateague mares in regard to activity budgets, although there was a trend for currently treated mares to spend more time in social behavior. Treatment did not affect spatial relationships between mares and stallions, social rank, or rates of aggression given or received (Powell, 1999).

The behavior of 30 mares in 13 harem groups on Shackelford Banks, North Carolina was observed during the non-breeding season. Mares were in various treatment statuses or had been untreated. Contracepted mares changed groups more often than untreated mares, visited more groups than untreated mares, and exhibited more reproductive interest. For both contracepted and untreated mares, the number of group changes and number of groups visited decreased with the number of years that mares were pregnant (Nuñez, et al, 2009).

The behavior of PZP-treated and untreated mares in 3 herds in Wyoming and Colorado were observed from April to October each year from 2003 – 2006. Treated mares received more reproductive behavior from stallions than untreated mares. Body condition was the strongest predictor of feeding, resting, maintenance, and social behaviors. Nursing mares had lower body condition than mares without a foal and there was no difference in body condition between treated and untreated mares (Ransom, et al, 2010).

Developmental and reproductive effects: Mares returned to fertility after discontinuation of PZP booster vaccinations, when treated for < 7 years; mares treated for 7 years did not return to fertility. Foals which were *in utero* at the time of treatment, matured, and gave birth to normal foals, as described below.

There are numerous reports detailing the contraceptive efficacy of PZP vaccination over the years. Initial studies used a priming injection of PZP/FCA while more recent studies used mFCA adjuvant as is used in the current registration. A study compared antibody titers from use of PZP/FCA with PZP/mFCA (Lyda, et al, 2005). It was found that PZP/mFCA had higher titers, although not statistically significant, than did PZP/FCA, indicating that PZP/mFCA should be as efficacious as the PZP/FCA adjuvant used in the earlier studies.

Analysis of fecal and urinary hormones has been used to monitor estrous cyclicity. Treatment for a single year did not appear to disrupt ovarian function, and fertility returned after discontinuation of treatment once antibody titers had fallen (Liu, et al, 1989). Ovulation rates and urinary estrogens declined with increasing years of treatment (Kirkpatrick, et al, 1995). For mares treated for 1, 2, or 3 years, the return to fertility was 100%, 100%, and 69%, respectively (n=53). For mares treated for 4 or 5 years (n=5), the return to fertility was 100%. No mares treated for 7 years returned to fertility (n=5). It took a longer time for mares to return to fertility the more years that they had been treated (Kirkpatrick and Turner, 2002).

In another study, fecal hormones from Assateague mares were monitored for 2 years. Mares were either being currently treated with PZP or had previously been treated with PZP; untreated controls were not available. All mares showed some evidence of cyclicity, but there was ovulatory failure (increased total estrogen excretion that was not followed by an increase in luteal protestagen) in both currently treated mares (2/3) and previously treated mares (3/9). The study authors concluded that the anovulatory state was episodic with variable durations (Powell and Monfort, 2001).

The incidence of seasonal births (April, May, and June) was calculated for PZP-treated foals on Assateague Island National Seashore for the years 1990 – 2002 (Kirkpatrick and Turner, 2003). Fecal and urinary hormones were monitored to determine pregnancy status in order to detect

early neonatal loss. The incidence of foals born in season was 76% for untreated mares (69/91) compared to 65% for treated mares (50/77). For mares foaling within 1 year of treatment (ineffective contraception), 69% of foals were born in season (20/29). For mares treated for longer than 2 years and then withdrawn from treatment, 62% foaled in season (30/48). Differences between treated groups and untreated mares were not statistically significant.

Mares which were vaccinated while pregnant have foaled normally and their foals, if untreated, have in turn foaled normally (Kirkpatrick and Turner, 2002). This is probably because there is not significant passage of maternal antibodies through the equine placenta.

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APPENDIX

Training Requirements from MIRD 47859806:

Application of ZonaStat-H is restricted to trained applicators. Applicators will be instructed in specific safety precautions to prevent accidental dermal or ocular exposure or needle stick. Precautions required of applicators include:

1. "One-hand" insertion of needle into adjuvant vial and replacement of plastic safety cover over needle;
2. Proper disposal of used needles and darts in sharps containers;
3. Proper disposal of syringes in clearly marked "Biohazard" bags;
4. Use of high-quality glass syringes to prevent breakage;
5. Wearing of latex or vinyl examination gloves during all operations in which accidental dermal exposure could occur, including washing of mixing syringes; and
6. Washing site of needles stick or cut with soapy water and disinfection of wound with alcohol or other disinfectant or antiseptic.

DATE OUT: 12/08/2010

SUBJECT: **PRODUCT CHEMISTRY REVIEW OF MP [ ] EP [X]**
DP BARCODE No.: D370437 **Symbol No.:** 86833-R
PRODUCT NAME: ZONASTAT-H
COMPANY: Humane Society of the United States
FOOD USE [ ] NON-FOOD USE [X] INTEGRATED FORMULATION [ ]
PCC: 176603 **DECISION No.** 420440 **ACTION CODE:** R110

FROM: Debra Rate
Alternative Risk Integration and Assessment (ARIA) Team
Risk Integration, Minor Use and Emergency Response Branch (RIMUERB)
Registration Division (RD; 7505P)

THROUGH: Shyam Mather
Product Chemistry Team Leader
Technical Review Branch/RD (7505P)

John Redden, Team Leader
ARIA/RIMUERB/RD (7505P)

TO: Jennifer Gaines/Kable (Bo) Davis, RM 07
Insecticide/Rodenticide Branch (IRB)/RD (7505P)

INTRODUCTION:

The registrant has submitted a registration application for the new restricted-use end-use product (EP) ZONASTAT-H. The EP is intended to be used as a contraceptive treatment to control wild horses and burros. The active ingredient (AI) is the porcine derived, glycoprotein ZP3. In support of the application, the applicant has submitted for review, product chemistry studies corresponding to guideline 830 series group A & group B (MRID No. 47859802). The registrant has also submitted a proposed CSF for basic formulation dated 09/16/2009 and the proposed product label. ARIA/RD has been asked to determine the acceptability of the product chemistry data submitted for the proposed end-use product and determine the acceptability of the proposed basic CSF.

SUMMARY OF FINDINGS

1. The proposed end-use product is a restricted-use product that can only be administerd by certified applicators. The proposed product contains the glycoprotein ZP3 (unregistered source) as the AI with a label claim nominal concentration of 0.071% with a protein content no less than 100 µg AI/0.5 mL product. The toxicity review was conducted on and the proposed label claims that the end-use product is a two component

product of two vials, one containing the AI as listed on the CSF, the other containing an adjuvant emulsion.

2. The proposed CSF for basic formulation (dated 09-16-2009) is not filled out completely or correctly. The nominal concentration of the active ingredient as expressed on the CSF does not concur with the product label claim nominal concentration. The CSF only reports the AI component. Because the end-use product is a mixture of two components that must be used in conjunction, the CSF must contain the components of both vials to be mixed. The CSF is not in compliance with PR Notice 91-2. Only those inert ingredients present in the AI vial of the end-use product have been approved by the Agency for the proposed uses (IIAB, 10-06-09).

3. The certified limits proposed by the registrant for the active ingredient are not based on the standard certified limit table set forth in 40CFR§158.350(b)(2). The registrant has provided justifications (via e-mail with reviewer) for the proposed certified limits. The data and justifications submitted corresponding to guidelines 830.1750 (Certified Limits) satisfy the product chemistry data requirements of 40CFR§158.32 [MRID No. 47859802]. The CSF must be revised accordingly. The justifications provided by the registrant to the reviewer are detailed in the Confidential Appendix.

4. The data submitted corresponding to guidelines 830.1600 (description of material used to produce the product), 830.1650 (description of formulation process), 830.1670 (discussion on the formation of impurity) and 830.1750 (certified limits) satisfy the data requirements of 40CFR §158.325, §158.335, §158.340 and 158.350 respectively. The formulation / isolation of the AI (ZP3) is described in the Confidential Appendix.

5. The product chemistry data submitted corresponding to the guidelines 830 series group B (physical-chemical properties) satisfy the data requirements of 40CFR §158.310(e). A signed self-certification statement was provided for the physical-chemical properties. Although it says that the product is stable for up to 2 years, this data must be submitted to the Agency, or a new storage stability study and corrosion characteristics study must be completed and submitted to the Agency for review upon completion [MRID No. 47859802].

CONCLUSIONS:

ARIA has reviewed the product chemistry data submitted for the proposed end-use product and has concluded that:

1. The proposed CSF for basic formulation (dated 09-16-2009) is unacceptable. A revised CSF must be submitted to the Agency clearing up the discrepancies as listed in Finding 2 and the Confidential Appendix.

2. The data submitted corresponding to guidelines 830.1600 (description of materials used to produce the product), 830.1650 (description of formulation process), and

830.1670 (discussion on the formation of impurity), and 830.1750 (certified limits) are acceptable. The justifications provided for the proposed wider certified limits for a few of the inert ingredients are acceptable.

3. The data submitted corresponding to guidelines 830.1800 (enforcement analytical method) does not satisfy the guideline requirements for the traditional end-use product. However, based on the manufacturing procedure and formulation process, as well as the label (which will be revised to clearly indicate the batch number from which the sample vial was produced and clearly express a defined expiration date, for this product), the method submitted to determine the total protein concentration of the batch (from which the single use sample vial will be produced) is adequate for Agency purposes.

4. The product chemistry data submitted corresponding to reference guidelines 830 series group B (physical-chemical properties) are acceptable, with the exception of 830.6317 (storage stability) and 830.6320 (corrosion characteristics). One year study results for the guidelines 830.6317 (1 year storage stability) and 830.6320 (corrosion characteristics) must be submitted to the Agency for evaluation. As the end-use product has a two year expiration date listed on the label, the data for which this expiration date is based may be sufficient to satisfy guideline requirements.

Product Chemistry Data (Series 830 group A & group B)

Subgroup A	Data Required Fulfilled	MRID No.
830.1550. Chemical Identity (basic CSF)	U	CSF (dated 09/16/09)
830.1600. Beginning Materials	A	47859802
830.1650. Formulation Process	A	47859802 (and e-mail communication)
830.1670. Discussion of Impurities	A	47859802
830.1700. Preliminary Analysis	NA	47859802
830.1750. Certified Limits (basic CSF)	U	47859802 (and e-mail communication)
830.1800. Enforcement Analytical Method	A	e-mail communication with reviewer

Subgroup B	Data Required Fulfilled	Value or Qualitative Description	MRID No.
830.6302. Color	A	Clear	47859802
830.6303. Physical State	A	Aqueous solution or powder	47859802
830.6304. Odor	A	Odorless	47859802
830.6314. Oxidation/Reduction Action	A	Denatured by acid or base, no incompatibility	47859802
830.6315. Flammability	A	Nonflammable (protein)	47859802
830.6316. Explodability	A	Not explosive (protein)	47859802
830.6317. Storage stability	I	Frozen liquid or (or powder in desicant) is viable for 2 years.	47859802
830.6319. Miscibility	A	Complete in water.	47859802
830.6320. Corrosion Characteristics	I	No Corrosive activity.	47859802
830.6321. Dielectric Breakdown Voltage	NA		
830.7000. pH	A	7.0-704	47859802
830.7100. Viscosity	A	Aqueous form is the same as water.	47859802
830.7000. Relative Density	A	1.0 in water	478586-02
830.7520. Particle size, fibre length, & diameter distribution	NA		

Explanations: A = The Requirements Were Fulfilled; N = The Requirements Were Not Fulfilled; NA = Not Applicable; G = Data Gap; U = Requires Upgrading; I = Incomplete or In Progress; W = Waived.

Confidential Appendix:

claimed confidential by submitter

claimed confidential by submitter

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

January 12, 2010

MEMORANDUM

Subject:	Name of Pesticide Product:	ZonaStat-H
	EPA File Symbol:	86833-R
	DP Barcode:	D370441
	Decision No.:	420440
	PC Code:	176603 Glycoprotein (Porcine zona pellucida)
	Action Code:	R110

From: Breann Hanson, Biologist BHanson
Alternative Risk Integration and Assessment (ARIA) Team
Risk Integration, Minor Use, Emergency Response Branch (RIMUERB)
Registration Division (RD) (7505P)

Through: John Redden, ARIA Team Leader JCR
RIMUERB/RD; 7505P

To: Jennifer Gaines, RM Team 07
Insecticide-Rodenticide Branch
RD; 7505P

Applicant: Humane Society of the United States
2100 L Street NW
Washington, DC 20037

FORMULATION FROM LABEL:

Active Ingredient:
176603 Glycoprotein (Porcine zona pellucida (PZP)) 100 µg*

* The product contains 100 µg of PZP per 0.5 mL dose

ACTION REQUESTED: The Product Manager requests: "Please review the enclosed acute tox study for the new a.i., porcine zona pellucida, to support the new registration of ZonaStat-H, a wild horse contraceptive. I have enclosed the study (MRID 47859803), CSF, cover letter, and label. Please let me know if you require any additional data. Thanks…"

BACKGROUND: The Humane Society of the United States (HSUS) (herein, the "registrant") has applied for registration of ZonaStat-H, EPA File Symbol: 86833-R, for use as a contraceptive in feral and wild horses and burros. The product contains a new active ingredient, porcine zona pellucida (PZP), derived from a naturally-occurring animal glycoprotein, which has not yet been registered with the Agency. The registrant has requested an exemption from the acute toxicity testing requirements. The bases for exempting ZonaStat-H were listed in a submitted toxicology volume (MRID 47859803) and include: a) documented safety and history of use of the components comprising the vaccine; b) the biology and properties of ZonaStat-H, along with the nature and fate of the product's metabolites, do not suggest that the product is toxic or pathogenic; c) the method of delivery (by injection) limits route of exposure for target and non-target animals; and d) extensive field and laboratory data document the safety of the vaccine product.

Composition: ZonaStat-H is an emulsion consisting of two components: a) a naturally occurring, chemically unmodified glycoprotein, PZP, and b) an adjuvant. PZP induces little or no immune response unless administered with an adjuvant (Bhatnager etal., 1989). Adjuvants are associated with side effects including injection site reactions such as granulomas and sterile abscesses, systemic effects such as fever, lethargy, and loss of appetite, and sometimes autoimmune diseases (Hanly etal., 1997).

ZonaStat-H uses 2 adjuvants; Modified Freund's Complete Adjuvant (mFCA) for primer injections, and Freund's Incomplete Adjuvant (FIA) for booster injections. In a variety of studies FCA/FIA vaccines produced antibody levels higher than other adjuvants (please see MRID 47859803).

Although FCA and FIA are not approved by the Federal Drug Administration or U.S. Department of Agriculture for commercial vaccines, the data amassed by the registrant on treated horses do not support the negative results observed which preclude use in human vaccines. The registrant presumes this can be due to several reasons. First, the efficacy and the type and magnitude of side effects elicited vary with species, route of administration, and adjuvant (MRID 47859803). Reports of side effects associated with Freund's adjuvants are derived from studies on laboratory animals, which include mice, rats, hamsters, guinea pigs and rabbits. The side effects noted in the studies submitted by the registrant on horses infer the safety of using FCA/FIA. Secondly, dosages administered in studies that did report side effects were considered extremely high relative to body weight. The dosage for the proposed use on wild horses is minimal compared to doses tested on laboratory animals. Thirdly, the composition of mineral oils has changed significantly over the 40-50 years in which this research has been done (Lindblad, 2000). In more recent preparations, unsaturated and aromatic hydrocarbons have been

removed, leaving less reactive, longer-chain saturated hydrocarbons which are non-carcinogenic in mice via the dermal or inhalation route; non-mutagenic by Ames Test, and non-fetotoxic and non-tetratogenic in rats treated via oral gavage (Stewart-Tull, 1997). Finally, another concern with the use of adjuvants is the risk of aggravating autoimmune diseases associated with antigens that resemble host proteins. PZP, however, does not cross-react with any equine somatic tissues or protein hormones (Kirkpatrick etal. 1996).

Mechanism of Action: ZonaStat-H stimulates a classic humoral response; the antibodies interfere with fertilization by binding to glycoprotein receptors on thee non-cellular membrane that surrounds the egg of the treated animal, causing steric hindrance of the zona sperm receptor (MRID 47859803).

Fate Post-Injection: Following injection, hydrolysis occurs, and the metabolic products are excreted and eliminated from the body in forms that are indistinguishable from other metabolic products (i.e., water, lactic acid, urea) (MRID 47859803). PZP and the adjuvant antigens are not stored in body tissues, thereby eliminating the possibility of continued exposure of the target animal to the vaccine components, or of non-target animals and humans of exposure to the components. Digestion of vaccine components yields end products comprising amino acids and simple carbohydrates, which elicit no immune response and are bioinactive. Therefore, vaccine components are not transferred through food chains (MRID 47859803).

Method of Administration and Exposure Risk: ZonaStat-H is injected intramuscularly, at a volume of 1 mL, either by hand-held syringe, by syringe attached to a "jab-stick", or by syringe dart. The method of delivery ensures that the target animal receives no aerial, oral, ocular, or general dermal irritation; exposure to non-target animals and humans is "nearly zero" (MRID 47859803).

Potential incidental contact with the contents of unrecovered, non-discharged darts is possible; though "approximately 95% of all darts fired are recovered" (Kirkpatrick, 2008); reducing the number of darts remaining in the environment. The proposed label includes language that instructs applicators to attempt to recover all darts, even proposing that lost darts should be noted and marked with an attempt to recover them at a later period. Also, the darts do not discharge spontaneously or with incidental contact. The registrant states that "[s]triking, stepping on, jiggling, biting, or otherwise casually moving or contacting the dart will not discharge or release the contents of the dart" (MRID 47859803). Therefore, the potential for incidental/unintentional contact is low.

Potential oral consumption by a predator or scavenger is another possibility. However; as the product is broken down into amino acids and simple carbohydrates following ingestion, the product becomes physiologically inactive (Takashima etal., 2008). The inerts within the product are known to either pass through the digestive system without absorption or are broken down.

Potential exposure to the applicator via dermal, oral and/or ocular contact with the product is

possible during handling/loading of the product. Therefore, the registrant is requiring training and certification for applicators (the product is a *Restricted Use Pesticide*), as well as requiring protective clothing during the preparation of the product (gloves).

Field and Laboratory Data on the Safety of ZonaStat-H: Adverse reactions to ZonaStat-H may occur at injection sites; including sterile granulomas and draining abscesses. Draining abscesses are rare though seen more often in horses treated via darts than in horses treated by hand injection (MRID 47859803). In one field study, three visible abscesses out of 26 mares receiving 2-3 injections were observed; all draining from 6-9 days after treatment (Kirkpatrick etal., 1990). In another field study, 1841 dartings of 329 wild horses yielded 19 visible abscesses (1% of all dartings); all drained within 30 days after treatment (Kirkpatrick, 2007). In the same study among zoo animals, 16 abscesses were noted out of 1185 treatments (1.35% of all treatments); all drained and healed without incident. In a western wild horse field study, no visible abscesses were observed in 215 mares receiving the product via hand-injection (Turner etal., 1997). Another study of 15 mares hand-injected with the product resulted in only 1 visible abscess (following a booster injection), which drained without incident (Lyda etal., 2005). In that study, the injection sites of 50 mares treated with the product in 4 different formulations yielded a rate of abscesses of 8% over 12 weeks post-treatment; at 10 months 2 still had palpable subcutaneous abscesses. At 7 months post-treatment, muscle tissue disruption at the injection site was noted in 8 of 28 horses examined; 7 were considered "slight".

Based on the above information, injection site reactions are of little concern to the Agency. The reactions noted do not preclude registration based on injection site irritation.

RECOMMENDATIONS: The registrant's request for a waiver of acute oral, acute dermal, acute inhalation, primary eye irritation, primary dermal irritation and dermal sensitization studies has been reviewed and determined by ARIA to be acceptable. Based on the information provided by the registrant, the overall acute toxicity of this product is expected to be low.

Acute Oral Toxicity: Based on the information that once ingested, the product yields end products comprising amino acids and simple carbohydrates which elicit no immune response and are bioinactive, and the low probability of oral contact, the product may be placed into acute oral toxicity category IV.

Acute Dermal, Inhalation, Eye, and Skin Toxicity: Based on the slight chance of exposure to the product and due to the irritation noted in the field and laboratory studies, the product may be placed into toxicity category III for these routes of exposure.

Skin Sensitization: The product does not have to be labelled as a dermal sensitizer.

The acute toxicity profile for ZonaStat-H, EPA File Symbol: 86833-R is:

Acute oral toxicity	IV	Waived

Acute dermal toxicity	III	Waived
Acute inhalation toxicity	III	Waived
Acute eye irritation	III	Waived
Primary skin irritation	III	Waived
Dermal sensitization	Neg.	Waived

Note to RM: The proposed label does not contain any signal word or EPA recommended precautionary and first aid statements. This reviewer concurs with the proposed precautionary statements regarding accidental needle pricks and contact with mFCA, but the additional statements noted below are additionally recommended. This reviewer strongly recommends that the signal word CAUTION appear on the label.

LABELING: Based on the toxicity profile above, the following are the precautionary and first aid statements for this product as obtained from the Label Review System.

PRODUCT ID #: 086833-00001
PRODUCT NAME: ZonaStat-H

PRECAUTIONARY STATEMENTS

SIGNAL WORD: CAUTION

Hazards to Humans and Domestic Animals: Harmful if absorbed through skin or inhaled. Avoid contact with skin, eyes or clothing. Causes moderate eye irritation. Wash thoroughly with soap and water after handling and before eating, drinking, chewing gum, using tobacco or using the toilet. Remove and wash contaminated clothing before reuse. Avoid breathing spray mist.

First Aid:

If on skin:
-Take off contaminated clothing.
-Rinse skin immediately with plenty of water for 15-20 minutes.
-Call a poison control center or doctor for treatment advice.

If inhaled:
-Move the person to fresh air.
-If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably mouth-to-mouth if possible.
-Call a poison control center or doctor for further treatment advice.

If in eyes:
-Hold eye open and rinse slowly and gently with water for 15-20 minutes.
-Remove contact lenses, if present, after the first 5 minutes, then continue rinsing.
-Call a poison control center or doctor for treatment advice.

Have the product container or label with you when calling a poison control center or doctor or going for treatment. You may also contact 1-800-xxx-xxxx for emergency medical treatment information.

REFERENCES:

Bhatnager, P., S. Sehgal, S.K. Gupta, and B.S. Dunbar. 1989. Necessity of adjuvants for inducing effective antibody response to porcine zona pellucida antigens. *Experientia* 45:759-762.

Hanly, W.C., B.T. Bennet, and J.E. Artwohl. 1997. Overview of adjuvants. Pp. 1-8 in C.P. Petrie Smith, D. Jensen, T. Allen, and M. Kreger, eds., *Information Resources for Adjuvants and Antibody Production: Comparisons and Alternative technologies 1990-1997*. U.S. Department of Agriculture/ARS/NAL, Beltsville, Maryland.

Kirkpatrick, J.F. 2008. Recovery of darts containing porcine zona pellucida vaccine delivered to wild horses at three field sites. Unpublished report dated February 20, 2008.

Kirkpatrick, J.F, I.K.M. Liu, and J.W. Turner. 1990. Remotely-delivered immunocontraception in feral horses. *Wildlife Society Bulletin* 18:326-330.

Kirkpatrick, J.F., Turner, J.W., Liu, K.M., and Fayrer-Hosken, R.A. 1996. Applications of pig zona pellucida immunocontraception to wildlife fertility control. *J. Reprod. Fertil.* (Supple. 50): 183-189.

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Lindblad, E.B. 2000. Freund's adjuvants. *Methods in Molecular Medicine* 42:49-63.

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Stewart-Tull, D.E.S. 1997. The use of adjuvants in the production of polyclonal antisera to protein antigens. Pp. 989-993 in L.F.M. van Zutphen & M. Balls (eds.), *Animal Alternatives, Welfare and Ethics*. Elsevier, Amsterdam.

Takashima, A., and R.M. Wooten. 2008. Unpublished letter dated July 30, 2008.

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Turner, J.W., Jr., I.K.M. Liu, A.T. Rutberg, and J.F. Kirkpatrick. 1997. Immunocontraception limits foal production in free-roaming feral horses in Nevada. *Journal of Wildlife*. 61: 873-880.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C., 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of Advanced Request for Minor Use Designation for ZonaStat-H (Porcine Zona Pellucida [PZP]) Immunocontraceptive for Use in Wild Horses and Burros

FROM: David Donaldson, Economist
Economic Analysis Branch
Biological and Economic Analysis Division (7503P)

THRU: Timothy Kiely, Chief
Economic Analysis Branch
Biological and Economic Analysis Division (7503P)

TO: Elizabeth Leovey
Senior Advisor for PRIA Implementation
Office of the Director
Office of Pesticide Programs (7501P)

Product Review Panel: April 8, 2009

Summary and Conclusions

The Humane Society of the United States (HSUS) intends to submit a pesticide registration application for its product, ZonaStat-H, which is an immunocontraceptive product intended for use in feral and wild horses and burros. In their letter to the Agency of February 6, 2009 (HSUS 2009), the Society asked for advance guidance regarding a "minor use" designation of their product, as defined by the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), such that ZonaStat-H and associated filings will be deemed exempt from any registration service fees under the Pesticide Registration Improvement Act of 2007 (PRIA).

In this memorandum the Biological and Economic Analysis Division (BEAD) concurs with the HSUS on the designation of the potential registration of ZonaStat-H as a minor

use pesticide. BEAD agrees with the HSUS that ZonaStat-H will fill an unmet need in providing control of wild horse and burro populations. BEAD considers it unlikely, based on the status of HSUS as a non-profit organization and their stated plan to cover only the cost of production from the sale of ZonaStat-H, that HSUS will generate earnings sufficient to cover PRIA registration fees through the production and sale of ZonaStat-H. BEAD notes that the conventional pesticide PRIA registration fee of $376,000 would result in an approximate $25 per unit cost and that a biological pesticide PRIA registration fee of $16,500 would result in an approximate $1 per unit cost, assuming 3,000 units of annual production and that the cost of PRIA fees are evenly distributed over the first five years of production.

Minor Use Designation

FIFRA Subchapter II § 136 (ll) establishes two relevant criteria for minor use pesticide designation. The first is an economic incentive criterion where,

> "... the Administrator, in consultation with the Secretary of Agriculture, determines that, based on information provided by an applicant for registration or a registrant, the use does not provide sufficient economic incentive to support the initial registration or continuing registration of a pesticide for such use. ..."

Under the second criterion, one of the following four conditions must be met,

> "(A) there are insufficient efficacious alternative registered pesticides available for the use;
> (B) the alternatives to the pesticide use pose greater risks to the environment or human health;
> (C) the minor use pesticide plays or will play a significant part in managing pest resistance; or
> (D) the minor use pesticide plays or will play a significant part in an integrated pest management program. ...."

HSUS asserts that ZonaStat-H meets the economic incentive criterion for a minor use pesticide such that production and sale of ZonaStat-H will not generate sufficient economic incentive to support PRIA registration fees. HSUS provided the following justifications:

> "... HSUS does not intend to offer the product for sale on the open market; rather, ZonaStat-H will be used by HSUS, governmental agencies such as the U.S. Bureau of Land Management, institutions, and non-profit groups to manage populations of wild horses and burros in the U.S. ..." HSUS further states, "... HSUS is a non-profit organization established in 1954 with a primary purpose of improving environmental conditions of animals, to the ultimate benefit of both the environment

> and the public. HSUS is not a commercial entity, nor could it be in its current form. It does not intend to become a commercial entity by marketing ZonaStat-H. Rather, HSUS intends to use ZonaStat-H as a tool to control, and to assist other agencies and non-profit organizations in controlling herds of wild horses and burros that are currently not adequately managed."

Based on the status of HSUS as a non-profit with the mission of improving animal welfare, BEAD agrees with HSUS that it is unlikely that production and sale of ZonaStat-H will result in appreciable earnings for the HSUS. At full production, the HSUS intends to produce a maximum of 3,000 units of ZonaStat-H annually at a targeted cost of production of $40 per unit. HSUS indicates that it will not sell or distribute ZonaStat-H at a price greater than the cost of production.

The PRIA registration fee for conventional pesticides is $376,000 and the PRIA fee for biological pesticides is $16,500. BEAD is not certain which PRIA registration fee would apply to the registration of ZonaStat-H. However, if PRIA registration fees are evenly distributed over the first five years of production, and the HSUS produces 3,000 units per year, the increase in cost for a conventional pesticide registration for ZonaStat-H resulting from PRIA registration fees would be $25 per unit or an additional 60%. The increase in cost for a biological pesticide registration for ZonaStat-H resulting from PRIA registration fees would be approximately $1 per unit or 3%.

HSUS further provides that, in line with conditions A, B, C, and D from the second FIFRA minor use pesticide criterion, ZonaStat-H fills an unmet need in controlling wild horse and burro populations and that insufficient efficacious alternative registered pesticides are available for wild horse and burro control; the alternatives to ZonaStat-H pose greater risks to the environment; ZonaStat-H will play a significant part in managing pest resistance; and ZonaStat-H will play a significant part in integrated pest management programs.

Based on information provided by HSUS and corroborated by the U.S. Bureau of Land Management (BLM) and Government Accountability Office (GAO), BEAD agrees with HSUS that ZonaStat-H meets condition A of criterion two and that there are insufficient efficacious alternative registered pesticides available for the control of wild horses and burros. Since only one of the conditions of criterion two must be met for a minor use designation to be made, and because BEAD is most suited to evaluate condition A, we have not evaluated conditions B, C, or D.

BLM (2009) indicates that Federal protection and a lack of natural predators have resulted in significant increases in wild horse and burro herd populations, which exceed the BLM estimate of the appropriate population levels in BLM managed lands of 27,300. Current wild horse and burro control programs are unable to maintain appropriate populations. BLM removes excess wild horses and burros from the land and either transfers them to private ownership or maintains them in BLM holding facilities. Since 2001, more than 74,000 wild horse and burros were removed from BLM managed land,

and only about 46,400 were adopted or sold—in 2008 BLM was holding over 30,000 animals (GAO, 2008). Wild horses and Burros also pose problems on other public land including National Forests, National Parks, and state managed lands. Wild horses and burros have no natural predators and herd sizes can double in about four years. According to BLM, there is an explicit need to manage wild horse and burro populations because uncontrolled populations may lead to degradation of wildlife and native vegetation habitat, result in soil erosion, and lead to conflicts with other rangeland uses such as cattle grazing and recreation.

Removal and slaughter or removal and euthanization provide additional alternative options for wild horse and burro control. However, neither option is considered viable or is currently practiced by BLM or other agencies. No pesticide control is currently being used.

References:

BLM. 2009. Factsheet on Challenges Facing the BLM in its Management of Wild Horses and Burros, March 2009. U.S. Bureau of Land Management. Washington DC. http://www.blm.gov/wo/st/en/prog/wild_horse_and_burro/new_factsheet.html

GAO. 2008. Report to the Chairman, Committee on Natural Resources, House of Representatives, Effective Long-Term Options Needed to Manage Unadoptable Wild Horses, Bureau of Land Management, October 2008. U.S. Government Accountability Office. Washington DC. http://www.gao.gov/new.items/d0977.pdf

HSUS. 2007. Helping Hands, 2007 Annual Report. The Humane Society of the United States. http://www.hsus.org/about_us/annual_reports_financial/annual_reports_financial_statements.html

HSUS. 2009. Request for Minor Use Designation for ZonaStat-H (Porcine Zona Pellucida [PZP]) Immunocontraceptive for Use in Wild Horses and Burros. Letter to Elizabeth Leovey. February 6, 2009.

U. S. Environmental Protect Agency
Office of Prevention, Pesticides, and Toxic Substances (OPPTS)
1200 Pennsylvania Avenue, NW
Washington, DC 20460

DOCKET VERIFICATION AND CERTIFICATION FORM

For Internal OPPTS Use Only

Title of Action: Registration Applications: Pesticide Products

RIN #:	**Docket ID #:** EPA-HQ-OPP-2009-0800	**FRL#:**

Docket Title: ***Notice of Receipt of a Section 3 registration request for a new active ingredient, Porcine Zona Pellucid.***

Contact Information:	**Name:** ***Jennifer Gaines***	**Phone #:** ***703-305-5967***

Legacy Information:

Program Lead's Verification: I have reviewed the docket and verified the following:

- ☐ All of the documents identified in the attached Docket Index have been submitted to the appropriate Docket Manager for inclusion in the docket identified above.
- ☐ Documents containing copyrighted, CBI, or otherwise protected information have been identified to allow for "special" processing by the appropriate Docket Staff.
- ☐ The material has been assembled in a useable form to support the document being published in the FEDERAL REGISTER (FR).
- ☐ **COMMENTS: Estimated FR publication date is.**

.Date:9/13/2011	Initials: ***Jennifer Gaines***	Phone #: ***703-305-5967***

Docket Manager's Verification and Sign-off: I hereby confirm the following:

- ☐ The Docket ID # identified above matches our records.
- ☐ The documents identified in the attached Docket Index have been received by the OPP Docket Staff.
- ☐ The documents have been properly processed for inclusion in EPA FDMS Dockets, as appropriate.
- ☐ The documents either already are in the docket or are being processed for inclusion in the docket.

COMMENTS:

Date: 9/14/11	Signature: Arthur White	Phone #: 703 305-5805

Program Lead's Certification: I hereby certify that:

- ☐ I have completed the verification above.
- ☐ I have submitted to the Docket Manager all of the documents that I identified needed to be updated or added to the docket.
- ☐ I have obtained the Docket Manager's sign-off.
- ☐ The docket is complete and ready for public release.

COMMENTS:

Date: 9/13/2011	Signature: ***Jennifer Gaines***	Phone #: ***703-305-5967***

Attachment: List of Documents for Docket ID # EPA-HQ-OPP-2009-0800 as of September 13, 2011

	Document Title	Source Information: Author (Last, First Name); Organization; Citation	Date of Document	1) CBI 2) Other Protected, e.g., Studies 3) Copyrighted
1.	ZONASTAT-H: Immunocontraceptive Vaccine for Limiting the Population of Wild and Feral Horses and Burros	Farwell, Kit, HED, EPA	**7/8/2010**	
2.	Section 3 Request for Zonastat, a New Chemical Proposed for Use to Control Wild Horses and Burros	Anderson, Brian; Sankula, Sujatha, EFED, EPA	**10/4/2010**	
3.	Efficacy Review – ZonaStat-H	Gaines.Jennifer	**3/15/2011**	
4.	Review of Advanced Request for Minor Use Designation for	Donaldson, David; Kiely, Timothy, BEAD, EPA		

	ZonaStat-H (Porcine Zo Pellucide [PZP]) Immunocontraceptive for Use in Wild Horses and Burros			
5.	Factsheet on Challenges Facing the BLM in its Management of Wild Horses and Burros	Gorey, Tom, BLM	**9/29/2009**	
6.	Myths and Facts	Gorey, Tom, BLM	***8/2011***	
7.	Reversibility of action and safety during pregnancy of immunization against porcine zona pellucid in wild mares (*Eqqus caballus*).	Kirkpatrick, J.F. and A. Turner	***2002***	
8.	Effective Long-Term Options Needed to Manage Unadoptable Wild Horses	Nazzaro, Robin M., US Government Accountability Office (GAO)	***10/2008***	
9.	Evaluation of somatic and reproductive immunotoxic effects of the porcine zona pellucida vaccination.	Barber, Matthew R. and Fayrer-Hosken, Richard A.		

Outline of a Proposal to the United States Environmental Protection Agency
Office of Pesticide Programs
Registration Division

from

The Humane Society of the United States
700 Professional Drive
Gaithersburg, Maryland 20879

Regarding the Registration of ZONASTAT-H, a Porcine Zona Pellucida Immunocontraceptive Vaccine for limiting the populations of wild and feral horses (*Equus caballus*) and burros (*Equus asinus*)

March 4, 2008

I. Product Description

Chemical Name

Antigen: Porcine Zona Pellucida

Adjuvants: Modified Freund's Complete Adjuvant
Modified Freund's Incomplete Adjuvant

Common Name: PZP

Description

Antigen:

Porcine zona pellucida (PZP) is a complex of four different acidic glycoproteins, ZP1 (80,000-90,000KD), ZP2 (60,000-65,000 KD), ZP3 (55,000 KD), and ZP4 20,000-25,000KD). PZP consists of approximately 70-80% ZP3, which itself has two components (α and β) (Dunbar, Liu et al. 1981; Hedrick and Wardrip 1987; Wassarman 1988).

Adjuvants:

Modified Freund's Complete Adjuvant (mFCA) is a yellow, oily bacterial suspension that consists of 85% Drakeol 5 NF, 15% Arlacel A (emulsifier), and 0.1% killed, dry cells of *Mycobacterium butyricum*. Modified Freund's Incomplete Adjuvant (mFIA) is identical to mFCA but lacks the bacterial cell component.

Pesticide Type: Inhibition of conception in wild and feral horses (*Equus caballus*) and burros (*Equus asinus*)

U.S. Producer

Antigen: Science and Conservation Center, Billings, Montana

Adjuvants: EMD Biosciences, Inc., San Diego, California

Product: ZonaStat-H (100 µg PZP antigen dissolved in 0.5 mL PBS, emulsified by the end user in 0.5 mL mFCA or mFIA adjuvant following procedures described in Kirkpatrick et al. (1990))

Mode of Action: When injected into a female horse, ZonaStat-H stimulates the production of anti-zona pellucida (ZP) antibodies. These antibodies bind to the native ZP glycoproteins surrounding the egg of the target female, alter their conformation, and block sperm attachment (Henderson, Hulme et al. 1988; Skinner, Prasad et al. 1996).

Packaging & Storage: PZP antigen dissolved in phosphate buffer solution will be packaged in screw-top plastic vials containing single 0.5 mL doses and stored frozen. The frozen antigen expires 2 years after freezing. After defrosting, it expires after 24 hours.

II. Use Patterns and Formulations

Application Sites: The use of ZonaStat-H is limited to female feral and wild horses and burros, which are defined as free-roaming horses or burros, privately or publicly owned, that are capable of doing environmental damage.

Methods and Rate of Application: After emulsification of the antigen solution and the adjuvant in the field, using two glass syringes connected by luer lock (Kirkpatrick, Liu et al. 1990), ZonaStat-H is injected intramuscularly in hip or gluteus muscles by hand-held syringe, syringe mounted on a jabstick, or by syringe dart fired from a CO2 or cartridge-powered projection system.

The initial treatment (priming dose) consists of the PZP/MFCA emulsion, typically followed two to four weeks later by one booster of the PZP/MFIA emulsion. Thereafter, boosters of PZP/MFIA are delivered annually to maintain contraceptive efficacy.

Although ZonaStat-H is safe to administer at any time of the year, it is most effective when a booster is delivered one to three months prior to the beginning of the breeding season in seasonally breeding wild or feral horses or burros. A single priming dose of PZP/MFCA is also effective at

a reduced level if delivered one to three months prior to the beginning of the breeding season in seasonally breeding wild or feral horses or burros.

III. Manufacture & Quality Control

Methods of production:

Antigen: The PZP antigen is produced following the methods of Dunbar et al. 1980 (Dunbar, Wardrip et al. 1980). Briefly, porcine ovaries are collected from freshly slaughtered female pigs at USDA-inspected slaughterhouses, and frozen immediately. Oocytes are extracted from the ovaries using a rotary-ganged razor blade device and washed with a buffered salt solution through a series of nylon screens, the last of which (74μm) traps the oocytes but permits dissolved proteins, erythrocyes, and other small debris particles to pass through. The isolated oocytes are then gently homogenized in buffered salt solution, and the zonae pellucidae collected on a 50μm screen and repeatedly washed. The isolated zonae are then heat-solubilized at 70°C for 30 minutes in phosphate buffer solution (PBS), and diluted to concentrations of approximately 5,000 zonae per 0.5 mL dose. The ZP solution is then frozen until use.

Adjuvant: Adjuvants (MFCA and MFIA) are purchased commercially from CalBiochem in 10 mL ampules.

Analysis: Protein concentration of the antigen is determined using a Bio-Rad DC protein assay, with Sigma's bovine serum albumen protein used to establish the standard curve. Qualitative analysis is done by electrophoresis, using the BioRad mini-Protean II cell system. The gel is developed with Bio-Rad Silver Stain Plus kit. Each gel is run with a prestained SDS-PAGE standard in the low range (approximately 18-106 KD).

Safety testing: Ovaries are obtained from USDA-inspected slaughterhouses. Pathogenic bacterial screening is conducted for each batch on a blood agar plate. PZP is not sterile, and there are occasional non-pathogenic gram positive rods in low numbers. Batches have been sent for viral screening to the USDA laboratory in Ames, Iowa, but no viral presence has been detected to date.

IV. Target Animal Safety

Autoimmune disease risk – All available evidence indicates that immune responses to PZP occur solely and uniquely in the ovary. In immunocytochemical studies, antibodies produced by rabbits injected with PZP did not bind or react to any of 14 horse and dog tissue types, including brain, heart, lung, kidney, liver, bladder, stomach, small intestine, large intestine, muscle, skin, spleen, pancreas or lymph node tissues (Barber and Fayrer-Hosken 2000). Radioimmunoassays had previously shown that antibodies produced by rabbits in response to PZP injections failed to bind or react to any of 22 fluid and tissue types in pigs except for ovarian tissue (Palm, Sacco et al. 1979).

Injection site reactions – Sterile granulomas (typically ~25mm in diameter) occur commonly at injection sites of horses that have received PZP/FCA or PZP/FIA injections delivered remotely by dart. Draining abscesses at the injection site are rare, though slightly more common in horses that are treated by dart than in horses that are treated by hand injection. In the initial field studies at Assateague Island National Seashore, three abscesses (10-25 mm diameter) were observed among 26 mares receiving 2-3 injections of PZP in FCA or FIA; all drained from 6 to 9 days after treatment (Kirkpatrick, Liu et al. 1990). As of July 2007, 1,841 dartings with 65-100 μg PZP/FCA or PZP/FIA of 329 individual horses at 4 locations have yielded 19 total abscesses (1%), ranging from 25-50 mm in diameter; all drained within 30 days (Kirkpatrick, unpublished data). No abscesses were observed in 215 mares hand-injected with PZP/FCA and PZP/FIA in two western wild horse populations, nor were injection-site marks observed in subsequent field observations (Turner, Liu et al. 1997; Turner, Liu et al. 2001). A study of 15 captive mares hand-injected with an initial shot of 100 μg PZP in modified Freund's Adjuvant (mFA) followed by a booster of 100 μg PZP in FIA resulted in 1 abscess (following a booster injection), which drained without incident (Lyda, Hall et al. 2005).

Safety to pregnant animals – There is abundant evidence that PZP injections have no effect on ongoing pregnancies. In the initial Assateague trials, all 14 mares known to have been pregnant at the time of treatment with 65-100 μg PZP/FCA or FIZ successfully produced foals, and all 11 mares known to have been pregnant in captive trials using 65-100 μg PZP in MFA/FIA successfully produced foals, all of which survived to weaning the following autumn (Kirkpatrick, Liu et al. 1990; Lyda, Hall et al. 2005). On Assateague, there were no differences in survival rates of foals born to treated and untreated mares, and PZP treatment of pregnant mares did not affect the fertility of their female offspring (Kirkpatrick and Turner 2002; Kirkpatrick and Turner 2003).

Longevity and body condition of treated mares – On Assateague Island, mares treated with PZP show better body condition and survive longer than mares not treated with PZP. New, older age classes (>21 years) of mares on Assateague Island began to appear ten years after the onset of PZP treatments in the herd; mean age of death of mares treated with vaccine for $\geq$ 3 yrs (19.9 yrs) was significantly greater than untreated mares (6.4 years), mares treated $\leq$ 3 yrs (10.2 yrs) and stallions (10.3 yrs) (Turner and Kirkpatrick 2002; Kirkpatrick and Turner 2007). Body condition also improved in the 10 years following beginning of PZP treatments for all animals except lactating mares (Turner and Kirkpatrick 2002).

Reversibility and ovarian effects – Contraception in horses treated with two initial doses of 65-100μg PZP emulsified in Freund's Complete Adjuvant (FCA; initial priming dose) or Freund's Incomplete Adjuvant (FIA; boosters) followed by annual boosters is fully reversible after up to five consecutive years of treatment, although mares treated for 4 or 5 years may experience a delay in return to fertility (Kirkpatrick and Turner 2002). Ovulation rates, as evidenced by pregnancies or luteal phase iPdG patterns, are 73% after one year of treatment, 56% after 3 consecutive years of treatment, and 10% after 7 consecutive years of treatment (Kirkpatrick, Naugle et al. 1995). In burros, full reversibility has been demonstrated after two years of treatment (Turner, Liu et al. 1996).

V. Environmental Impacts

Oral activity -- As a naturally occurring animal protein that is used in human and animal food and food byproducts, there is no expectation that PZP would be physiologically or immunologically active when consumed orally (Miller 1997). This is confirmed by experimental studies. Rabbits fed adjuvanted PZP proteins showed no anti-PZP antibody titers, nor did control and treatment groups differ in the number or stage of embryos produced (Barber and Fayrer-Hosken 2000). Likewise, mice and rabbit fed PZP directly in PBS, or fed PZP in alginate microspheres with or without a cholera-toxin adjuvant, showed no significant rise in anti-PZP antibody titers, nor did they show any difference in litter size (Martin, Suckow et al. 2006).

PZP in ZonaStat-H is a naturally occurring glycoprotein extracted by simple physical and chemical processes from the ovaries of pigs slaughtered for human consumption. It is not modified from the natural product, nor is it incorporated into any agent or vector. Therefore PZP is not transmissible by any mechanism, nor is it effective as a contraceptive or physiologically active if consumed as food.

Environmental recovery – Because ZonaStat-H is delivered in very small quantities (100 μg antigen in1 mL liquid) directly to target animals via hand-injection or darting, there is very little risk of direct exposure by non-target animals or release into the environment. Darts that miss their targets do not discharge their contents when hitting other substrates (unless they strike a tree trunk or similarly rigid surface, which is exceedingly rare). The dart-delivery protocol requires that every attempt be made to recover all darts fired, whether they strike their target or not. Dart recovery rates on three sites are very high, varying narrowly from 94-96%: 1,115 recovered of 1,185 fired on Assateague Island National Seashore, 301 recovered of 313 fired on Cape Lookout National Seashore, and 140 recovered of 146 fired at Little Book Cliffs Wild Horse Range, Colorado (J. Kirkpatrick, pers. comm., compiled from darting records).

VI. Product Efficacy

Liu et al. (1989) first demonstrated in principle the efficacy of PZP by suppressing fertility with multiple hand injections of PZP in captive mares. They also demonstrated that anti-PZP antibody titers of 64% or greater predicted effective contraception. Kirkpatrick et al. (1990) remotely injected 26 mares on Assateague Island with a priming dose of 65-100 μg PZP in FCA

and either one or two boosters of PZP in FIA at three week intervals; only one of the 26 treated mares produced foals the following year. Of the 26 treated mares, 14 were boosted again a year later with a single remotely delivered dart containing PZP in FIA. Only 1 of the boosted mares produced a foal the following year (Kirkpatrick, Liu et al. 1991) Follow-up studies over the next six years at Assateague demonstrated foaling rates of approximately 4% among PZP-treated mares vs. 46% in untreated mares (Kirkpatrick, Naugle et al. 1995).

Comparable results were seen in tests of two-injection PZP protocols on free-roaming feral burros at Virgin Islands National Park, St. Johns, VI. In that study, 0 of 13 females darted with a priming dose of 65-100 μg PZP in FCA and a booster of 65-100 μg PZP in FIA produced foals in the period 12-24 months after treatment, while 6 of 11 control females gave birth in that time period (Turner, Liu et al. 1996). (Feral burros on the Virgin Islands are not seasonal breeders, and some were pregnant at the time of treatment).

Hand-injection of a priming dose of 65-100 μg PZP in FCA followed by hand-injection of 65-100 μg in FIA has also been carried out on western wild horses. One study showed foaling rates of approximately 5% among 44 treated mares; a second study showed foaling rates of 13% among 78 treated mares; in both cases, approximately half of untreated controls produced foals (Turner, Liu et al. 1997; Turner, Liu et al. 2001). Variation in efficacy between studies can be entirely accounted for by sampling error (i.e., 95% confidence intervals for the proportion of treated animals reproducing overlap for all studies), although differences in injection quality, nutritional condition, and other variables might affect contraceptive effectiveness.

Freund's modified adjuvant (MFA) has been substituted for FCA in titer trials of captive mares. No significant difference was seen in antibody titers between mares hand-injected with 65-100 μg PZP in MFA followed by a booster of 65-100 μg in FIA and mares treated with 65-100 μg PZP in FCA followed by a booster of 65-100 μg in FIA, and 7 of 8 PZP/MFA mares remained above the contraceptive titer threshold after 10 months (Lyda, Hall et al. 2005).

As described in detail in section IV above("*Reversibility and ovarian effects*"), consecutive years of treatment may suppress conception for more than one year after treatments stop; however partial reversibility is attainable.

VII. Background and Rationale

The responsibility for managing populations of wild horses (*Equus caballus*) and burros (*Equus asinus*) in the U.S. depends on who owns the land they occupy. On private and state-owned lands, wild horses typically are categorized as estrays, and become the responsibility of state agencies (typically departments of livestock) to dispose of when conflicts arise. The most prominent public controversies surrounding wild horses have arisen on federal land, however. The Department of Interior (DOI), the U.S. Forest Service (USFS), and the Department of Defense (DOD) all manage properties that support wild horses and burros, with each agency operating under a different legal mandate.

Most federal responsibility for managing wild horses and burros lies with the DOI's Bureau of Land Management (BLM) which, under the Free-Roaming Wild Horse and Burro Act (the "Act"; P.L. 92-195, amended), manages all wild horses and burros on BLM land and cooperates with the USFS to manage wild horse territories on national forests land (GAO 1990). Passed unanimously in 1971, with overwhelming public support, the Act was intended to end a long history of brutality towards wild horses on public lands (Ryden 1999; Rutberg 2003). The Act bans the killing, harassment, or removal of wild horses on BLM and USFS lands, and assigns to the BLM the authority and responsibility for managing these horses as components of the public lands. Currently, the BLM manages approximately 28,500 wild horses and 2,900 burros in 199 herd management areas (HMA), which occupy more than 34 million acres of public lands.

Populations of wild horses or burros also occupy a number of properties managed by the National Park Service (NPS, in the DOI). Under its 2006 Management Policies, the NPS classifies wild horses and burros as "exotic species" or :"feral livestock.," unless protected by park-specific legislation. As such, they are subject to management, removal, and/or destruction if they are "unacceptably impacting park resources" (NPS 2006). At a number of units, including Assateague, Cape Lookout, and Cumberland Island National Seashores, Ozark National Recreational Area, and Virgin Islands National Park, wild horses and burros are managed by law as a "historic resource," but even at these units concerns continue to exist about impacts on park resources. In others, such as Grand Canyon National Park and Mojave National Preserve, horses and burros have been systematically removed or eliminated because of what the agencies judge to be adverse impacts on natural resources (NPS 2002).

Because BLM and USFS lands are generally managed under "multiple-use" policies, the federal agencies employ a NEPA-based public process to develop management plans that allocate resources among wild horses, wildlife, livestock growers, and other users of the public lands. For wild horses and livestock, this process results in the establishment of appropriate management levels (AML's). AML's set limits on the number of wild horses and livestock that can occupy a given HMA in order to maintain a sustainable level of productivity on the range and prevent environmental damage. Ranchers are responsible for maintaining livestock numbers at or below the AML set in their grazing permits, and the BLM is responsible for maintaining the AML for wild horses.

Wild horse populations can be extremely prolific. On Atlantic coastal barrier islands, they typically grow at about 10% per year (Zimmerman, Sturm et al. 2006). Published estimates of population growth in wild horse herds on Western public lands range from 15-27% per year, with a mean of about 20-21% (Eberhardt, Majorowicz et al. 1982; Berger 1986; Garrott, Siniff et al. 1991).

Although there is often controversy about the relative impact of wild horse and burro populations on the environments they occupy, a number of investigators have presented evidence that wild horse and burro populations that are allowed to grow without management controls may achieve densities that damage land and wildlife habitat, and bring about conflicts with livestock growers and recreational users. Some researchers have argued that at high densities, wild horses on Assateague Island, MD, destabilize and reduce the height of the dunes that protect the island by trampling and grazing intensively on dune grass (*Ammophila breviligulata*) and salt meadow hay

(*Spartina patens*) (Seliskar 2003; De Stoppelaire, Gillespie et al. 2004). In some instances, grazing by wild horses on barrier islands has been shown to reduce the biomass and productivity of marsh grass (*Spartina alterniflora*), shift species composition to the less palatable forage species *Distichlis spicata*, and change soil chemistry (Furbish and Albano 1994; Zimmerman, Sturm et al. 2006). Grazing by horses also influences the animal ecology of the marsh in complex ways, affecting diversity of shore birds and the density of colonial nesting birds, crabs, and salt marsh fishes (Levin, Ellis et al. 2002). Management of conflicts between wild horses and park visitors is a perpetual issue at Assateague Island National Seashore and other heavily visited barrier island parks, where wild horses habituated to human handouts loiter dangerously along roadsides and damage campsites. They may also nip and kick beachgoers while seeking refuges from biting flies on sandy beaches (Zimmerman, Sturm et al. 2006).

On rangelands in the western U.S., there are data indicating that wild horses influence the productivity, biomass, and species composition of rangeland plants, utilize scarce water resources, and compact soil. In one landscape-level study in the Great Basin, ending grazing by wild horses increased shrub and grass cover and species richness; excluding horses from water sources increased local plant species richness, percent plant cover, and abundance of grasses and shrubs (Beever and Brussard 2000; Beever, Tausch et al. 2007). Because of their effects on vegetation and soil, wild horses on these rangelands may also shape wildlife habitat and influence species composition of vertebrate and even invertebrate communities (Beever and Brussard 2000; Beever 2003; Beever and Brussard 2004; Beever and Herrick 2006). In New Zealand, high densities of wild horses have caused dramatic changes in grassland communities and damaged the habitat of rare plants (Rogers 1991). Wild horses can also cause soil compaction in intensively used areas, reducing the potential productivity of the habitat (Beever and Herrick 2006).

The diets of wild horses foraging on western rangelands overlap to a high degree with those of cattle, sheep, and elk, and therefore can compete directly with these animals for forage (Hansen and Clark 1977; Hanley and Hanley 1982; Beever and Brussard 2000; Beever 2003; Beever and Brussard 2004). Several studies have reported that ivestock owners experience significant economic costs when wild horses exceed BLM-established appropriate management levels (Hyde 1978). In a Wyoming case study, opportunity costs in terms of income losses to livestock owners caused by surplus wild horses were estimated at approximately $1900 per horse (Bastian, Van Tassell et al. 1999).

Wild burros typically occupy more arid habitats than wild horses. Consequently, burro impacts tend to be closest to water sources. In the Sonoran desert, burros used nearly 70% of available forage from their favored species within 0.5 km of a water source, and plant density and cover was sharply reduced for this and many other species in these areas (Hanley and Brady 1977). At Mojave National Preserve and other sites in the western U.S., federal management agencies including the NPS consider wild burros to be a threat to natural resources, most notably the desert tortoise and desert bighorn sheep, both of which are listed as threatened under the Endangered Species Act (NPS 2002). Consequently, thousands of burros have been removed from Mojave, Death Valley National Monument, and Grand Canyon National Park. Free-living burros are also being aggressively removed from islands, including the Galapagos, because of

concerns that they threaten imperiled endemic species of plants and animals, and may have been partly responsible for the extinction of others (Carrion, Donlan et al. 2007).

Options for managing wild horses and burros and controlling their impacts are extremely limited. Killing wild horses is deeply unacceptable to the public, and is illegal in most circumstances (GAO 1990). Currently, the only form of wild horse population management that is acceptable to the public is to gather wild horses from the range and remove some of them, either for adoption or public auction. Between 1971 and 2006, 267,000 horses were removed from the range, of which 217,000 have been adopted by members of the public through the BLM's Adopt-a-Horse-or-Burro program. In 2004, the BLM was further required by Congress to sell certain horses removed from the range; about 2,500 horses have been sold under that program.

However, there are serious concerns about the cost, effectiveness, and humaneness of gather-and-removal (GAO 1990). The cost of a gather typically exceeds $100,000, and the cost of gathering, handling, processing, transporting, holding, and adopting ranges from $800 to $1600 per horse (Godfrey and Lawson 1986; GAO 1990; Kirkpatrick 2005). Moreover, sale and adoption have historically proven to insufficient for disposing of wild horses removed from the range (GAO 1990). The BLM is currently holding more than 28,000 horses in long-term holding facilities, corrals, and other facilities. In FY 2006, BLM spent $19.6 million maintaining these horses, which constitutes 53% of the entire budget of the agency's wild horse and burro program. Because of the challenges that wild horses (especially older animals) pose to potential adopters, many of these horses have limited adoption prospects. Neglect, exploitation, and sale to slaughter of adopted wild horses have also been extensively documented, and the program remains controversial (GAO 1990; BLM 1997; Ryden 1999)

Because of the difficulties, expense, and inadequacy of gather and removal for managing wild horses, the BLM has been exploring and promoting research in wild horse fertility control since the 1970's (Kirkpatrick, Turner et al. 1982; NRC 1991; Plotka, Vevea et al. 1992; Kirkpatrick 2005). Section 3(b)1 of the Act specifically authorizes fertility control as a means to control wild horse populations. Early research focused on injections or implants of steroid hormones into both males and females; although some of these approaches proved pharmacologically successful, they were ultimately rejected because of logistical difficulties, concerns about secondary consumption by non-target animals, and humane considerations (Kirkpatrick 2005).

Consequently, research began to shift in the early 1990's to potential applications of the porcine zona pellucida (PZP) immunocontraceptive vaccine (Turner and Kirkpatrick 1991). This switch followed the demonstration that PZP effectively blocked conception in captive mares (Liu, Bernoco et al. 1989). As a naturally-occurring animal glycoprotein vaccine, PZP is effective when injected in microgram quantities, is biodegradable, and is not active when consumed orally (see below).

Beginning in 1988, field tests of PZP on wild horses began under the sponsorship of the National Park Service (NPS) at Assateague Island National Seashore (ASIS), MD (Kirkpatrick, Liu et al. 1990; Kirkpatrick, Naugle et al. 1995). As noted above, ASIS management became interested in wild horse contraception because an exponential increase in the size of the island's wild horse population was becoming associated with progressively more severe impacts on dune stability

and salt marsh physiography and ecology, and increased conflicts with island visitors (Kirkpatrick 1995). Because of agency-wide policies that discourage intensive management of park natural resources, ASIS staff sought a minimally-invasive method to control its wild horse population. As discussed below, the ASIS study has provided the richest, most comprehensive data set extant on PZP safety, efficacy, and population effects. The project has also contributed significantly toward mitigating NPS concerns regarding wild horse impacts.

Following the demonstration at ASIS that PZP was an effective horse contraceptive, the BLM signed a Memorandum of Agreement with The Humane Society of the United States (HSUS) to cooperate on research applying PZP to wild horses on western public lands (Kirkpatrick 2005; Appendix). Since that time, the BLM has provided more than $1 million in direct support for PZP wild horse research, and has participated in tests of PZP on a number of its herd management areas (Turner, Liu et al. 2002). In 2006, research had advanced sufficiently that the BLM signed a second MOU with HSUS, this time to move PZP fertility control towards widespread management use on wild horses on public lands (Appendix).

More than two decades of research on PZP have generated a comprehensive, well-documented record of safety and effectiveness in wild horses and burros. In addition, because it requires minimal or no handling of animals, PZP vaccination is extremely cost effective (Kirkpatrick 2005). An economic analysis conducted for the BLM indicated that PZP treatments would cut program costs by 21-27% through reductions in the number of gathers and the number of horses processed for adoption, effectively saving millions of dollars annually (Bartholow 2007). Because of its enormous potential for cost-effective problem-solving, the BLM, the USFS, and the NPS all have strong institutional interests in seeing PZP registered and made available for management use on wild horses.

VIII. Proposed registration

Full registration of ZonaStat-H is being sought. This drug has been used outside of the laboratory for over 20 years.

IX. Labeling Restrictions

Risk to non-target animals, and human applicators, is minimal. However, to mitigate risk to non-target animals, the following label requirements are proposed for ZonaStat-H

- Fully Registered Pesticide for use only by Certified Applicators or persons under their direct supervision and only for those uses covered by the Certified Applicator's certification.
- Applicators must obtain a certification from a certifying institution or agent approved and authorized by The Humane Society of the United States. The certifying institution or agent will follow standards and protocols developed by the Science and Conservation

Center, Billings, Montana, in consultation with The Humane Society of the United States[1].

- The permit applicant must identify the Certified Applicator who will apply the drug.
- Application of ZonaStat-H is limited to free-roaming horses and burros, privately or publicly owned, that are capable of causing environmental damage.
- Limitations on geographical use: None

X. References

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[1] The need for mandatory certification is driven by the degree of care needed in the preparation, handling, transportation, and delivery of ZonaStat-H.

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Morgan Lewis
COUNSELORS AT LAW

Kathleen M. Sanzo
Partner
202.739.5209
ksanzo@MorganLewis.com

September 17, 2009

VIA HAND DELIVERY

Meredith F. Laws
Chief, Insecticide-Rodenticide Branch
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 S. Crystal Drive
Arlington, Virginia 22202

John D. Hebert
Product Manager, Insecticide-Rodenticide Branch
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 S. Crystal Drive
Arlington, Virginia 22202

Re: Restricted Use Pesticide Application – ZonaStat-H

Dear Ms. Laws and Mr. Hebert:

On behalf of The Humane Society of the United States ("HSUS"), we hereby submit the enclosed registration application and its contents, which are intended to support the registration of ZonaStat-H (porcine zona pellucida) contraceptive for use in feral and wild horses and burros.

HSUS greatly appreciates the guidance that the registration division has provided in HSUS's preparation and compilation of the attached registration application and supporting documentation in furtherance of HSUS's ZonaStat-H product, which reflects a collaboration of research efforts over the past 20 years. HSUS believes ZonaStat-H will offer a meaningful and humane solution to the overpopulation of wild horses and burros.

I. Background

Environmental repercussions of wild horse and burro overpopulation can be extensive, and may include such changes as: destabilization and reduction in dune height (due to trampling and intensive grazing on grass and salt meadow hay) (Seliskar 2003; De Stoppelaire et al. 2004); reduction in biomass and productivity of marsh grass (Spartina alterniflora); a shift in species composition to a less palatable forage species (Distichlis spicata); and changes in soil chemistry (Furbish and Albano 1994; Zimmerman et al. 2006). Grazing by horses also influences the animal ecology of the marsh in complex ways, affecting diversity of shore birds and the density of colonial nesting birds, crabs, and salt marsh fishes (Levin, Ellis et al. 2002).

The HSUS has worked to supplement programs of the Department of Interior ("DOI"), the U.S. Forest Service ("USFS"), the Department of Defense ("DOD"), and the DOI's Bureau of Land Management ("BLM") to humanely support and control populations of wild horses and burros that occupy public lands. Because the mandates of these various governmental agencies are varied, and because no contraceptive product limiting population growth in wild horses and burros is available or has been approved, the animals can be subject to management, removal, and/or destruction.

As a vaccine derived from naturally-occurring, animal glycoprotein, PZP is effective when injected in microgram quantities, is biodegradable, and is not active when consumed orally. Potential applications of earlier versions of ZonaStat-H, the porcine zona pellucida ("PZP") immunocontraceptive vaccine, became a focus of research as a tool to manage populations of wild horses beginning in the late 1980's (Turner and Kirkpatrick 1991). Since that time, and as is reflected in the attached, more than two decades of continued research on PZP have generated a comprehensive, well-documented record of safety and effectiveness of this product in wild horses and burros.

II. Applicant and Contact Information

Consistent with the requirements of PR Notice 86-5, the mailing address for the applicant, HSUS, is as follows:

John W. Grandy, Ph.D.
Senior Vice President, Wildlife Programs
The Humane Society of the United States
2100 L Street, N.W.
Washington D.C. 20037

Please let us know if you have any questions regarding the enclosed application and accompanying materials.

Sincerely,

Kathleen M. Sanzo/akh

Kathleen M. Sanzo, Esq.
Counsel to HSUS

Attachments

cc: John W. Grandy, Ph.D.
Senior Vice President, Wildlife Programs
The Humane Society of the United States

Stephen Paul Mahinka, Esq.
Sharon A. Segal, Ph.D.
Alexis Reisin Miller, Esq.

Please direct all correspondence regarding this submission to counsel for HSUS, as follows:

Kathleen M. Sanzo, Esq.
Morgan, Lewis & Bockius LLP
1111 Pennsylvania Avenue, N.W.
Washington, D.C. 20004

III. Minor Use Pesticide –PRIA Registration Fee and Payment Not Applicable

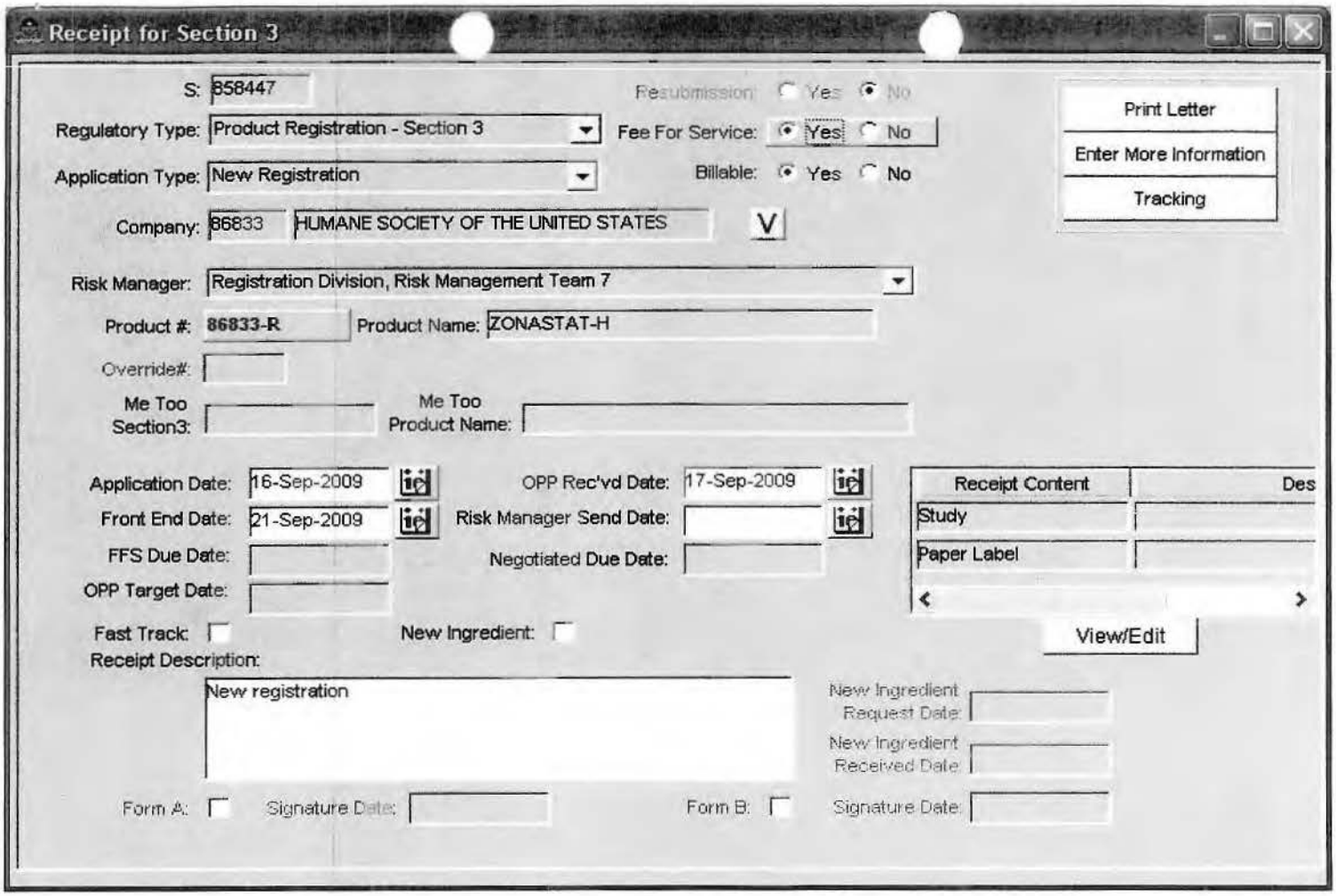
Pursuant to our exchanges with the Agency earlier this year, ZonaStat-H of PZP has been recognized as a "minor use pesticide" as that term is defined under the Federal Insecticide, Fungicide and Rodenticide Act of 1947 ("FIFRA"). Accordingly, pursuant to FIFRA Section 33(b)(7)(D), ZonaStat-H and any associated filings are exempt from any registration service fees under the Pesticide Registration Improvement Act of 2007 ("PRIA"). 7 U.S.C. § 136w-8(b)(7)(D).

IV. Materials Submitted

The enclosed submission contains eight (8) total volumes of supporting documentation

- Volume I – Administrative Materials
- 47859801 • Volume II – Product Efficacy
- 47859802 • Volume III – Product Identity and Composition
- 47859803 • Volume IV – Toxicology – Acute
- 47859804 • Volume V – Toxicology – Subchronic, Developmental and Reproductive Toxicity; Genotoxicity; Neurotoxicity; and Immunotoxicity
- 47859805 • Volume VI – Ecological Effects
- 47859806 • Volume VII – Human Exposure
- 47859807 • Volume VIII – Environmental Fate

Reference to no required registration fee.


Receipt for Section 3
S: 858447
Resubmission: Yes No
Regulatory Type: Product Registration - Section 3
Fee For Service: Yes No
Application Type: New Registration
Billable: Yes No
Company: 86833 HUMANE SOCIETY OF THE UNITED STATES
V
Print Letter
Enter More Information
Tracking
Risk Manager: Registration Division, Risk Management Team 7
Product #: 86833-R
Product Name: ZONASTAT-H
Override#:
Me Too Section3:
Me Too Product Name:
Application Date: 16-Sep-2009
OPP Rec'vd Date: 17-Sep-2009
Front End Date: 21-Sep-2009
Risk Manager Send Date:
FFS Due Date:
Negotiated Due Date:
OPP Target Date:
Receipt Content
Des
Study
Paper Label
Fast Track:
New Ingredient:
View/Edit
Receipt Description:
New registration
New Ingredient Request Date:
New Ingredient Received Date:
Form A:
Signature Date:
Form B:
Signature Date:

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

September 24, 2009

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MORGAN, LEWIS & BOCKIUS LLP
HUMANE SOCIETY OF THE UNITED STATES
1111 PENNSYLVANIA AVENUE, NW
WASHINGTON, DC 20004-

Report of Analysis for Compliance with PR Notice 86-5

Thank you for your submittal of 17-SEP-09. Our staff has completed a preliminary analysis of the material. The results are provided as follows:

Your submittal was found to be in full compliance with the standards for submission of data contained in PR Notice 86-5. A copy of your bibliography is enclosed, annotated with Master Record ID's (MRIDs) assigned to each document submitted. Please use these numbers in all future references to these documents. Thank you for your cooperation. If you have any questions concerning this data submission, please raise them with the cognizant Product Manager, to whom the data have been released.

United States Environmental Protection Agency
Office of Public Programs
Information Resources and Services Division
Information Services Branch

EPA

ADMINISTRATIVE NO(S).: 86833-R

PM: 7

CHEMICAL NO.: _____

The jacket for this action can be requested through the JACKETS system.

21-Day Screen Completed by Contractor

21-Day Expires on 10-2-09

Jacket # 86833-R
MRID# 478598

Content Screen: Recommended to Pass/Fail (Fail) multiple issues please see PAGE 3

86-5 Review: Passed/Failed/NA (Passed)

Transfer This Jacket to:

LINDA ARRINGTON

Miller, Alexis Reisin to: John Hebert 10/02/2009 02:34 PM
Cc: "Sanzo, Kathleen M.", "Segal, Ph.D., Sharon A."

John,

As we discussed this morning, the following responds to Kirk Clausen's questions regarding the ZonaStat-H submission.

1) We have attached a completed EPA form 8570-34.

Inert ingredient information may be entitled to confidential treatment

Please let us know if you have any questions or need anything further.

Best regards,

Alexis

Alexis Reisin Miller
Morgan, Lewis & Bockius LLP
1111 Pennsylvania Avenue, NW | Washington, DC 20004
Direct: 202.739.5390 | Main: 202.739.3000 | Fax: 202.739.3001
alexis.miller@morganlewis.com | www.morganlewis.com
Assistant: Althea L. Heard | 739-5849 | aheard@morganlewis.com

-----Original Message-----
From: Clausen.Kirk@epamail.epa.gov [mailto:Clausen.Kirk@epamail.epa.gov]

Sent: Thursday, September 24, 2009 2:02 PM
To: Sanzo, Kathleen M.
Cc: nair.sree@epa.gov
Subject: ZonaStat-H

Ms. Sanzo,

This is Kirk Clausen, EPA contractor. This email is in regards to your submission of ZonaStat-H (EPA Reg # 86833-R). We have found the following deficiencies with the application package:

1) Form 8570-34 (Certification with respect to citation of data) is missing
2) The active ingredient (Porcine zona pellucida) is not listed on the CSF; additionally, we cannot seem to find a CAS# for this ingredient
3) Cannot find inert ingredients on approved list. Background information on these ingredients may be required for this product.
4) The active ingredient is not listed as a % of the total substance on the label.

These deficiencies have been approved by the EPA. You may email these corrections to me or fax them to 703.305.5060/Attn: Kirk Clausen. If you have any questions you may contact me at 703.347.8784 or you may email me.

Thank you,

Kirk Clauen
Environmental Analyst
Macfadden
EPA contract

ZonaStat-H Vial Label (updated with 0.1%)(10.02.09).pdf

EPA cert for citation of data PZP 10 09 (EXECUTED).pdf

ZonaStat-H Bag Label (updated with 0.1%)(10.02.09).pdf

ZonaStat-H Package Insert (updated with 0.1%)(10.02.09).pdf

Form Approved OMB Nos. 2070-0060; 2070-0057; 2070-0107; 2070-0122; 2070-0164

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
1200 Pennsylvania Avenue, N.W.
WASHINGTON, D.C. 20460

Certification with Respect to Citation of Data

Applicant's/Registrant's Name, Address, and Telephone Number	EPA Registration Number/File Symbol
The Humane Society of the United States, 2100 L Street NW Washington, DC 20037, 202-452-1100	
Active Ingredient(s) and/or representative test compound(s)	**Date**
Porcine Zona Pellucida (PZP)	October 2, 2009
General Use Pattern(s) (list all those claimed for this product using 40 CFR Part 158)	**Product Name**
Minor Use, Restricted Use	ZonaStat-H

NOTE: If your product is a 100% repackaging of another purchased EPA-registered product labeled for all the same uses on your label, you do not need to submit this form. You must submit the Formulator's Exemption Statement (EPA Form 8570-27).

☐ I am responding to a Data-Call-In Notice, and have included with this form a list of companies sent offers of compensation (the Data Matrix form should be used for this purpose).

SECTION I: METHOD OF DATA SUPPORT (Check one method only)

☐ I am using the cite-all method of support, and have included with this form a list of companies sent offers of compensation (the Data Matrix form should be used for this purpose).

☑ I am using the selective method of support (or cite-all option under the selective method), and have included with this form a completed list of data requirements (the Data Matrix form must be used).

SECTION II: GENERAL OFFER TO PAY

[Required if using the cite-all method or when using the cite-all option under the selective method to satisfy one or more data requirements]

☐ I hereby offer and agree to pay compensation, to other persons, with regard to the approval of this application, to the extent required by FIFRA.

SECTION III: CERTIFICATION

I certify that this application for registration, this form for reregistration, or this Data-Call-In response is supported by all data submitted or cited in the application for registration, the form for reregistration, or the Data-Call-In response. In addition, if the cite-all option or cite-all option under the selective method is indicated in Section I, this application is supported by all data in the Agency's files that (1) concern the properties or effects of this product or an identical or substantially similar product, or one or more of the ingredients in this product; and (2) is a type of data that would be required to be submitted under the data requirements in effect on the date of approval of this application if the application sought the initial registration of a product of identical or similar composition and uses .

I certify that for each exclusive use study cited in support of this registration or reregistration, that I am the original data submitter or that I have obtained the written permission of the original data submitter to cite that study.

I certify that for each study cited in support of this registration or reregistration that is not an exclusive use study, either: (a) I am the original data submitter; (b) I have obtained the permission of the original data submitter to use the study in support of this application; (c) all periods of eligibility for compensation have expired for the study; (d) the study is in the public literature; or (e) I have notified in writing the company that submitted the study and have offered (i) to pay compensation to the extent required by sections 3(c)(1)(F) and/or 3(c)(2)(B) of FIFRA; and (ii) to commence negotiations to determine the amount and terms of compensation, if any, to be paid for the use of the study.

I certify that in all instances where an offer of compensation is required, copies of all offers to pay compensation and evidence of their delivery in accordance with sections 3(c)(1)(F) and/or 3(c)(2)(B) of FIFRA are available and will be submitted to the Agency upon request. Should I fail to produce such evidence to the Agency upon request, I understand that the Agency may initiate action to deny, cancel or suspend the registration of my product in conformity with FIFRA.

I certify that the statements I have made on this form and all attachments to it are true, accurate, and complete. I acknowledge that any knowingly false or misleading statement may be punishable by fine or imprisonment or both under applicable law.

Signature	Date	Typed or Printed Name and Title
	October 2, 2009	John Grandy, Ph.D., Senior VP, Wildlife Programs

PRIA 2 – 21 Day Content Screen Review Worksheet

(EPA/OPP Use Only)

3/23/09

21 Day Screen Start Date: 9-17-09

Experts In-Processing Signature: MF HARRINGTON Date 9-23-09 Fee Paid: Yes __

Division management contacted on issues No_____ Yes _____ Date _____

EPA Reg. Number: 86833-R	EPA Receipt Date: 9-17-09

	Items for Review			Yes	No	N/A*
1	**Application Form** (EPA Form 8570-1)(link to form) signed & complete including package type			X		
2	**Confidential Statement of Formula** all boxes completed, form signed, and dated (EPA Form 8570-4) (Link to form)			X		
	a) All inerts (link to http://www.epa.gov/opprd001/inerts/), including fragrances, approved for the proposed uses (see Footnote A)	**yes**	**no**			
3	**Certification with Respect to Citation of Data** (EPA Form 8570-34) (Link to form) completed and signed (N/A if 100% repack)				X	
	Certificate and data matrix consistent					
	If applicant is relying on data that are compensable, is the offer to pay statement included. (see Footnote B)	**yes**	**no**			
	If applicable, is there a letter of Authorization for exclusive use only.					
4	**Formulator's Exemption Statement** (EPA Form 8570-27) (Link to form) completed and signed (N/A if source is unregistered or applicant owns the technical)					X
5	**Data Matrix** (EPA Form 8570-35) (Link to form) both internal and external copies (PR 98-5) (Link to PR 98-5) completed and signed (N/A if 100% repack)			X		
	a) Selective Method (Fee category experts use)	**yes**	**no**			
	b) Cite-All (Fee category experts use)					
	c) Applicant owns all data (Fee category experts use)					
6	**5 Copies of Label (link to http://www.epa.gov/oppfead1/labeling/lrm/) (Electronic labels on CD are encouraged and guidance is available)(link to** http://www.epa.gov/pesticides/regulating/registering/submissions/index.htm#labels)			X		

7	**Is the data package consistent with PR Notice 86-5** (link to PRN 86-5)	X		
8	**Notice of Filing (link to http://www.epa.gov/pesticides/regulating/tolerance_petitions.htm) included with petitions (link to http://www.epa.gov/pesticides/regulating/tolerances.htm)**			X
9	**If applicable for conventional applications, reduced risk rationale (link to http://www.epa.gov/opprd001/workplan/reducedrisk.html)**			X
10	**Required Data (link to http://www.epa.gov/pesticides/regulating/data_requirements.htm) and/or data waivers. See Footnote C.**			
	a) List study (or studies) not included with application			

Comments: KC 9/23

☐ Study associated w/ jacket has passed 86-5 review

☐ Form 8570-34 is missing

☐ Submitter has requested additional time to send in needed documentation (see attached coresponsance, pg before CSF envelope)

Fail

MRID 478598

* N/A – Not Applicable

Footnotes

A. During the 21 day initial content review, all CSFs will be reviewed to determine whether all inerts listed, including fragrances, are approved for the proposed uses. If an unapproved inert is identified, the applicant must either 1) resolve the inert issue by, for example, removing the inert, substituting it with an approved inert, submitting documentation that EPA approved the inert for the proposed pesticidal uses, correcting mistakes on the CSF, etc. or 2) provide the data to support OPP approval of the inert or 3) withdraw the application. Removing or substituting an inert ingredient will require a new CSF and may require submission of data. <u>All information, forms, data and documentation resolving the inert issue must have been received by the Agency or the application withdrawn within the 21 day period, otherwise, the Agency will reject the application as described below.</u>

To successfully complete this aspect of the 21 day initial content screen, applicants are **strongly encouraged** to verify that all inert ingredients have been approved for the application's uses **even if a product is currently registered** by consulting the inert Web

site [link to http://www.epa.gov/opprd001/inerts/lists.html] and if the inert is not approved, to **obtain the necessary inert approval prior to submitting an application to register a pesticide product containing that inert ingredient.** Some inert ingredients are no longer approved for food uses or certain types of uses. The name and/or CAS number on a CSF must match the name and CAS number on this web site. Simple typographical errors in the name or CAS number have resulted in processing delays.

If an inert is not listed on the inert ingredient web site and the applicant believes that the inert has been approved, the applicant should contact the Inert Ingredient Assessment Branch (IIAB) at inertsbranch@epa.gov and resolve the issue. Copies of the correspondence with IIAB resolving the issue should accompany the application. All new inerts except PIP inerts are reviewed by IIAB. The IIAB should also be contacted for any questions on what supporting data needs to be submitted for and the Agency's inert review process. Questions on PIP inerts should be directed to the Chief of Microbial Pesticides Branch [Link to http://www.epa.gov/oppbppd1/biopesticides/contacts_bppd.htm].

When a brand, trade, or proprietary name of an inert ingredient is listed on a CSF, additional information such as an alternate name of the inert, CAS number or other information [link to http://www.epa.gov/opprd001/inerts/tips.pdf] must also be included to enable the Agency to determine if it has been approved. Each component of an inert mixture (including a fragrance) must be identified. In some cases, the supplier of the mixture or fragrance may need to provide this information to the Agency. Prior to the Agency's receipt of an application, applicants must arrange with a proprietary mixture or fragrance supplier to provide the component information to the Agency or promptly upon EPA's request. If the inert ingredients in a proprietary blend (including fragrances) cannot or are not identified or provided within the 21-day content review period, the Agency will reject the application.

During the 21 day content review, applicants should submit information to the individual identified by the Agency when the applicant is informed of an unapproved inert.

Unapproved Inerts Identified on CSFs

All applications except conventional new products and PIPs

Once an unapproved inert is identified on a CSF, the Agency will contact the applicant with the following options:

1. Correct the application by, for instance, correcting the inert's identity or CAS number, providing documentation that the inert has been approved, or removing the unapproved inert from the CSF or replacing it with one that is approved for the application's uses; or

2. Submit the information and data needed for the Agency to approve the unapproved inert. If this option is selected and implemented, the Agency may request an extension in the PRIA decision review timeframe to accommodate the inert review/approval process;

3. Withdraw the application (the Agency retains 25% of the full fee for the fee category estimated); or

If none of these options is selected and implemented by the applicant within the 21 day content review period, the Agency will reject the application and retain 25% of the full fee of the category identified.

Conventional New Product Applications

When the Registration Division identifies an unapproved inert on a CSF with an application for a new product that the applicant has not identified as requiring an inert approval (R311, R312 or R313), it will contact the applicant with the following options:

1. Correct the application by, for instance, correcting the inert's identity or CAS number, providing documentation that the inert has been approved, or removing the unapproved inert from the CSF or replacing it with one that is approved for the application's uses; or

2. Submit the information and data needed for the Agency to approve the unapproved inert, including any required petition to establish or amend a tolerance or exemption from a tolerance. (This option may change the PRIA category for the application, which could require a longer decision review time and a larger fee. If additional fees are due, they must be received by the Agency within the 21 day content review period.)

3. Withdraw the application (the Agency retains 25% of the full fee for the fee category estimated); or

If none of the above options is selected and implemented during the 21-day content-review period, the Agency will reject the application and retain 25% of the appropriate fee for the new product-inert approval category.

PIP Applications

When the Biopesticide and Pollution Prevention Division identifies an unapproved inert on a PIP CSF and a request to approve the inert does not accompany the application, it will contact the applicant with the following options:

1. Correct the application by, for instance, correcting the spelling or name of the inert to that in 40 CFR 174, or providing documentation that the inert has been approved; or

2. Submit the information and data needed for the Agency to approve the unapproved inert. If an inert ingredient tolerance exemption petition is required, the petition must be received by the Agency and the B903 fee paid within the 21 day period. If this option is selected and implemented, the Agency will discuss harmonizing the timeframe for both actions.

3. Withdraw the application (the Agency retains 25% of the full fee for the fee category estimated); or

If none of the above options is selected and implemented during the 21 day content review period, the Agency will reject the application and retain 25% of the fee.

B. A policy on documentation of offers to pay is still being developed, however, for a me-too or fast track (similar/identical) new product, R300 or A530, an application without the necessary authorizations of offers to pay will be placed into either R301 or A531. The Agency recommends that authorizations of offers to pay be submitted with other PRIA applications to avoid delays in the Agency's decision.

C. Biopesticide applicants are advised to contact the Agency and discuss study waivers prior to submitting their application to the Agency. Documentation of such discussions should be submitted with the study waiver.

ZonaStat-H
Miller, Alexis Reisin
to:
Kirk Clausen
10/01/2009 04:52 PM
Show Details

Kirk,

We are preparing a response to the issues you note below, and were wondering if there was any way we could provide this additional information next week rather than tomorrow, so that we can ensure our response and the materials we provide address the items noted below.

Please advise at your earliest convenience.

Best regards,

Alexis

Alexis Reisin Miller
Morgan, Lewis & Bockius LLP
1111 Pennsylvania Avenue, NW | Washington, DC 20004
Direct: 202.739.5390 | Main: 202.739.3000 | Fax: 202.739.3001
alexis.miller@morganlewis.com | www.morganlewis.com
Assistant: Althea L. Heard | 739-5849 | aheard@morganlewis.com

-----Original Message-----

From: Clausen.Kirk@epamail.epa.gov [mailto:Clausen.Kirk@epamail.epa.gov]
Sent: Thursday, September 24, 2009 2:02 PM
To: Sanzo, Kathleen M.
Cc: nair.sree@epa.gov
Subject: ZonaStat-H

Ms. Sanzo,

This is Kirk Clausen, EPA contractor. This email is in regards to your submission of ZonaStat-H (EPA Reg # 86833-R). We have found the following deficiencies with the application package:

1) Form 8570-34 (Certification with respect to citation of data) is missing
2) The active ingredient (Porcine zona pellucida) is not listed on the CSF; additionally, we cannot seem to find a CAS# for this ingredient
3) Cannot find inert ingredients on approved list. Background information on these ingredients may be required for this product.
4) The active ingredient is not listed as a % of the total substance on the label.

These deficiencies have been approved by the EPA. You may email these corrections to me or fax them to 703.305.5060/Attn: Kirk Clausen. If you have any questions you may contact me at 703.347.8784 or you may email me.

Thank you,

Kirk Clauen
Environmental Analyst
Macfadden
EPA contract

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

September 21, 2009

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

OPP Decision Number: D-420440
EPA File Symbol or Registration Number: 86833-R
Product Name: ZONASTAT-H
EPA Receipt Date: 17-Sep-2009
EPA Company Number: 86833
Company Name: HUMANE SOCIETY OF THE UNITED STATES

KATHLEEN M. SANZO
MORGAN, LEWIS & BOCKIUS LLP
HUMANE SOCIETY OF THE UNITED STATES
1111 PENNSYLVANIA AVENUE, NW
WASHINGTON, DC 20004-

SUBJECT: Receipt of Registration Application and Minor Use Fee Waiver Request

Dear Registrant:

The Office of Pesticide Programs has received your application for registration and minor use fee waiver request. If you submitted data with this application, the results of the PRN-86-5 screen will be communicated separately. During the administrative screen, the Office of Pesticide Programs has determined that this Action is subject to a Pesticide Registration Service Fee as defined in the Pesticide Registration Improvement Act.

The Action has been identified as Action Code: R110
NEW AI;NON-FOOD USE;INDOOR;

Your request for waiver has been forwarded for review. You will be notified in writing when a determination is made regarding your request. If the determination indicates that payment is due, you will receive instructions for submitting payment at that time.

If you have any questions, please contact the Pesticide Registration Service Fee Ombudsman, at (703) 305-6249.

Sincerely,

Teresa Downs

Front End Processing Staff
Information Technology & Resources Management Division

Fee for Service {858447L~

This package includes the following

- ◉ New Registration
- ○ Amendment

for Division

- ○ AD
- ○ BPPD
- ◉ RD

☑ Studies? ☑ Fee Waiver?

☐ volpay % Reduction: 100

Fee exemption requested.

Risk Mgr. 7

Receipt No. S- 858447

EPA File Symbol/Reg. No. 86833-R

Pin-Punch Date: 9/17/2009

☐ This item is NOT subject to FFS action.

Action Code:

Requested: None

Granted: R110

Amount Due: $ 209.475

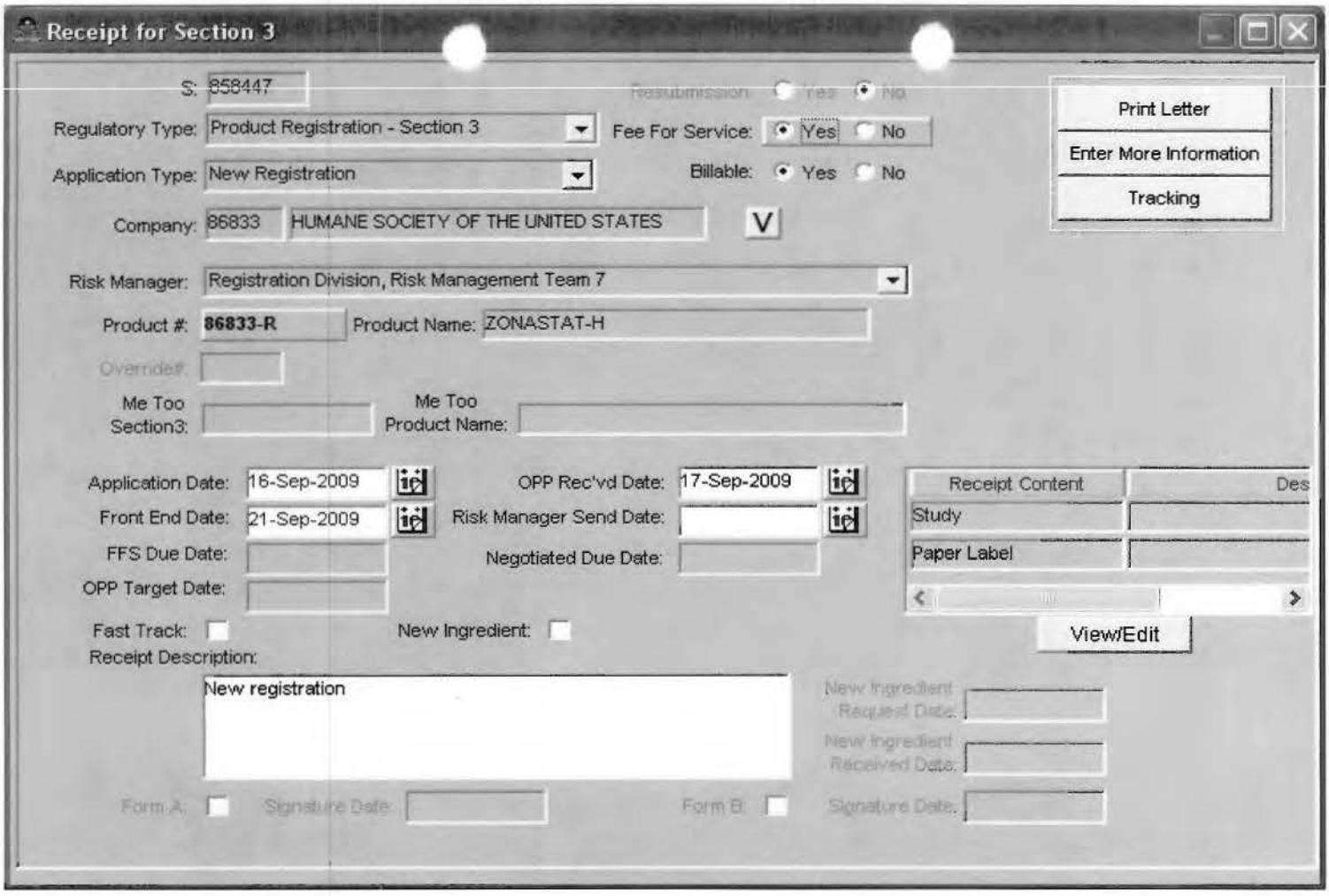
Parent/Child Decisions:

☐ Inert Cleared for Intended Use ☐ Uncleared Inert in Product

Reviewer: RK Date: 9-21-09

Remarks:

Fee exemption requested under FIFRA 33(b)(7)(D).


Receipt for Section 3
S: 858447
Resubmission: Yes No
Regulatory Type: Product Registration - Section 3
Fee For Service: Yes No
Application Type: New Registration
Billable: Yes No
Company: 86833 HUMANE SOCIETY OF THE UNITED STATES
Print Letter
Enter More Information
Tracking
Risk Manager: Registration Division, Risk Management Team 7
Product #: 86833-R
Product Name: ZONASTAT-H
Override#:
Me Too Section3:
Me Too Product Name:
Application Date: 16-Sep-2009
OPP Rec'vd Date: 17-Sep-2009
Front End Date: 21-Sep-2009
Risk Manager Send Date:
FFS Due Date:
Negotiated Due Date:
OPP Target Date:
Receipt Content
Des
Study
Paper Label
Fast Track:
New Ingredient:
View/Edit
Receipt Description:
New registration
New Ingredient Request Date:
New Ingredient Received Date:
Form A:
Signature Date:
Form B:
Signature Date:

FEE FOR SERVICE

Please read instructions on reverse before completing form. Form Approved, OMB No. 2070-0060 Print Form

EPA

ed States
Environmental Protection Agency
Washington, DC 20460

[X] Registration
[ ] Amendment
[ ] Other

OPP Identifier Number

Application for Pesticide - Section I

1. Company/Product Number	2. EPA Product Manager	3. Proposed Classification
86833-R	John Hebert	[ ] None [X] Restricted
4. Company/Product (Name)	**PM#**	
Humane Society of the United States/ZonaStat-H	7	

5. Name and Address of Applicant *(Include ZIP Code)*

The Humane Society of the United States
2100 L Street NW
Washington, DC 20037

[ ] *Check if this is a new address*

6. Expedited Review. In accordance with FIFRA Section 3(c)(3)(b)(i), my product is similar or identical in composition and labeling to:

EPA Reg. No. _____

Product Name _____

Section - II

[ ] Amendment - Explain below.
[ ] Resubmission in response to Agency letter dated _____
[ ] Notification - Explain below.
[ ] Final printed labels in response to Agency letter dated _____
[ ] "Me Too" Application.
[ ] Other - Explain below.

Explanation: Use additional page(s) if necessary. (For section I and Section II.)

Section - III

1. Material This Product Will Be Packaged In:

Child-Resistant Packaging	Unit Packaging	Water Soluble Packaging	2. Type of Container
[ ] Yes* [X] No	[X] Yes [ ] No	[ ] Yes [X] No	[ ] Metal [X] Plastic [ ] Glass [ ] Paper [ ] Other (Specify) _____
* *Certification must be submitted*	If "Yes" Unit Packaging wgt. 0.5 g / No. per container Variable	If "Yes" Package wgt / No. per container	

3. Location of Net Contents Information	4. Size(s) Retail Container	5. Location of Label Directions
[X] Label [X] Container	0.5 g	[ ] On Label [X] On Labeling accompanying product

6. Manner in Which Label is Affixed to Product [X] Lithograph / Paper glued / Stenciled [ ] Other _____

Section - IV

1. Contact Point *(Complete items directly below for identification of individual to be contacted, if necessary, to process this application.)*

Name	Title	Telephone No. (Include Area Code)
John W. Grandy, Ph.D.	Senior Vice President	202-452-1100

Certification

I certify that the statements I have made on this form and all attachments thereto are true, accurate and complete. I acknowledge that any knowingly false or misleading statement may be punishable by fine or imprisonment or both under applicable law.

6. Date Application Received (Stamped)

2. Signature	3. Title	4. Typed Name	5. Date
[signature]	Senior Vice President, Wildlife Programs	John W. Grandy	09/16/09

Morgan, Lewis & Bockius LLP
1111 Pennsylvania Avenue, NW
Washington, DC 20004
Tel: 202.739.3000
Fax: 202.739.3001
www.morganlewis.com

Morgan Lewis
COUNSELORS AT LAW

Kathleen M. Sanzo
Partner
202.739.5209
ksanzo@MorganLewis.com

September 17, 2009

VIA HAND DELIVERY

Meredith F. Laws
Chief, Insecticide-Rodenticide Branch
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 S. Crystal Drive
Arlington, Virginia 22202

John D. Hebert
Product Manager, Insecticide-Rodenticide Branch
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 S. Crystal Drive
Arlington, Virginia 22202

Re: Restricted Use Pesticide Application – ZonaStat-H

Dear Ms. Laws and Mr. Hebert:

On behalf of The Humane Society of the United States ("HSUS"), we hereby submit the enclosed registration application and its contents, which are intended to support the registration of ZonaStat-H (porcine zona pellucida) contraceptive for use in feral and wild horses and burros.

HSUS greatly appreciates the guidance that the registration division has provided in HSUS's preparation and compilation of the attached registration application and supporting documentation in furtherance of HSUS's ZonaStat-H product, which reflects a collaboration of research efforts over the past 20 years. HSUS believes ZonaStat-H will offer a meaningful and humane solution to the overpopulation of wild horses and burros.

I. Background

Environmental repercussions of wild horse and burro overpopulation can be extensive, and may include such changes as: destabilization and reduction in dune height (due to trampling and intensive grazing on grass and salt meadow hay) (Seliskar 2003; De Stoppelaire et al. 2004); reduction in biomass and productivity of marsh grass (Spartina alterniflora); a shift in species composition to a less palatable forage species (Distichlis spicata); and changes in soil chemistry (Furbish and Albano 1994; Zimmerman et al. 2006). Grazing by horses also influences the animal ecology of the marsh in complex ways, affecting diversity of shore birds and the density of colonial nesting birds, crabs, and salt marsh fishes (Levin, Ellis et al. 2002).

The HSUS has worked to supplement programs of the Department of Interior ("DOI"), the U.S. Forest Service ("USFS"), the Department of Defense ("DOD"), and the DOI's Bureau of Land Management ("BLM") to humanely support and control populations of wild horses and burros that occupy public lands. Because the mandates of these various governmental agencies are varied, and because no contraceptive product limiting population growth in wild horses and burros is available or has been approved, the animals can be subject to management, removal, and/or destruction.

As a vaccine derived from naturally-occurring, animal glycoprotein, PZP is effective when injected in microgram quantities, is biodegradable, and is not active when consumed orally. Potential applications of earlier versions of ZonaStat-H, the porcine zona pellucida ("PZP") immunocontraceptive vaccine, became a focus of research as a tool to manage populations of wild horses beginning in the late 1980's (Turner and Kirkpatrick 1991). Since that time, and as is reflected in the attached, more than two decades of continued research on PZP have generated a comprehensive, well-documented record of safety and effectiveness of this product in wild horses and burros.

II. Applicant and Contact Information

Consistent with the requirements of PR Notice 86-5, the mailing address for the applicant, HSUS, is as follows:

John W. Grandy, Ph.D.
Senior Vice President, Wildlife Programs
The Humane Society of the United States
2100 L Street, N.W.
Washington D.C. 20037

Please direct all correspondence regarding this submission to counsel for HSUS, as follows:

Kathleen M. Sanzo, Esq.
Morgan, Lewis & Bockius LLP
1111 Pennsylvania Avenue, N.W.
Washington, D.C. 20004

III. Minor Use Pesticide –PRIA Registration Fee and Payment Not Applicable

Pursuant to our exchanges with the Agency earlier this year, ZonaStat-H of PZP has been recognized as a "minor use pesticide" as that term is defined under the Federal Insecticide, Fungicide and Rodenticide Act of 1947 ("FIFRA"). Accordingly, pursuant to FIFRA Section 33(b)(7)(D), ZonaStat-H and any associated filings are exempt from any registration service fees under the Pesticide Registration Improvement Act of 2007 ("PRIA"). 7 U.S.C. § 136w-8(b)(7)(D).

IV. Materials Submitted

The enclosed submission contains eight (8) total volumes of supporting documentation.

- Volume I – Administrative Materials
- Volume II – Product Efficacy
- Volume III – Product Identity and Composition
- Volume IV – Toxicology – Acute
- Volume V – Toxicology – Subchronic, Developmental and Reproductive Toxicity; Genotoxicity; Neurotoxicity; and Immunotoxicity
- Volume VI – Ecological Effects
- Volume VII – Human Exposure
- Volume VIII – Environmental Fate

Please let us know if you have any questions regarding the enclosed application and accompanying materials.

Sincerely,

Kathleen M. Sanzo/ARH

Kathleen M. Sanzo, Esq.
Counsel to HSUS

Attachments

cc: John W. Grandy, Ph.D.
Senior Vice President, Wildlife Programs
The Humane Society of the United States

Stephen Paul Mahinka, Esq.
Sharon A. Segal, Ph.D.
Alexis Reisin Miller, Esq.

Form Approved OMB No. 2070-0060

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
401 M Street, S.W.
WASHINGTON, D.C. 20460

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DATA MATRIX

Date	EPA Reg No./File Symbol	Page 1 of 2
Applicant's/Registrant's Name & Address Humane Solciety of the united States, 2100 L St. NW, Washington, DC 20037	Product ZonaStat-H	

Ingredient

Guideline Reference Number	Guideline Study Name	MRID Number	Submitter	Status	Note
Series 810	Product Performance Test Guidelines			PL	
Series 830	Product Properties Test Guidelines			PL	
Series 835	Fate, Transport, and Transportation Guidelines			PL	
Series 850	Ecological Effects Test Guidelines			PL	
Series 870	Health Effects Test Guidelines			PL	
Series 875	Occupational and Residential Exposure Test Guidelines			PL	

Signature	Name and Title	Date
	John W. Grandy, Ph.D. Senior Vice President of Wildlife Prog.	09/16/09

EPA Form 8570-35 (9-97) Electronic and Paper versions available. Submit only Paper version.

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Ingredient

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Series 810	Product Performance Test Guidelines			PL	
Series 830	Product Properties Test Guidelines			PL	
Series 835	Fate, Transport, and Transportation Guidelines			PL	
Series 850	Ecological Effects Test Guidelines			PL	
Series 870	Health Effects Test Guidelines			PL	
Series 875	Occupational and Residential Exposure Test Guidelines			PL	

Signature	Name and Title	Date
	John W. Grandy, Ph.D. Senior Vice President of Wildlife Prog.	09/16/09

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Series 835	Fate, Transport, and Transportation Guidelines			PL	
Series 850	Ecological Effects Test Guidelines			PL	
Series 870	Health Effects Test Guidelines			PL	
Series 875	Occupational and Residential Exposure Test Guidelines			PL	

Signature	Name and Title	Date
	John W. Grandy, Ph.D. Senior Vice President of Wildlife Prog.	09/16/09

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